THE ENDOCRINE CONTROL AND MANIPULATION OF HAIR FOLLICLE ACTIVITY IN THE CASHMERE GOAT.

BY

Pamela Dicks B.Sc. Agric. Hons (Animal Sc.).

A thesis submitted to the Faculty of Science the University of Edinburgh for the degree of Doctor of Philosophy 1992

Declaration

I Pamela Dicks (neē Lynch) hereby declare that this thesis has been composed by myself and has not been presented or accepted in any previous application for a degree, and is a record of work carried out by myself unless stated otherwise; all quotations have been distinguished by quotation marks and all sources of information acknowledged.

signature

PAMELA DICKS

name

SUMMARY

The cashmere goat has an annual cycle of hair growth and moulting, which is associated with changes in photoperiod.

The first experiment was designed to determine if slow release melatonin implants could be used to delay the spring moult and initiation of hair folicle activity, as has been reported in mink and Blue- foxes, and to investigate other endocrine changes associated with the onset of the moulting period. Adult and juvenile cashmere goats were implanted with melatonin from mid-December until May. The adult goats became photorefractory to the continuous release of melatonin, and exhibited advances in the onset of moulting and the seasonal rise in plasma prolactin concentration, when compared to the control animals. The juveniles did not differ from the controls in any of the parameters measured. The data were not consistent with the hypothesis that melatonin implants could be used to delay the spring moult and reactivation of the hair follicles in the cashmere goat.

Another group of goats was treated with melatonin implants in April. They demonstrated a significant depression in plasma prolactin concentration compared to the controls.

In the second experiment the asociation between the increase in plasma prolactin concentration and the onset of moulting and hair follicle activity was investigated. Prior to the experiment a dose response study of the dopamine-agonist bromocriptine was conducted to establish the dose of bromocriptine required to suppress plasma prolactin concentration to base-line levels for 14 days. Groups of adult female goats were treated with either exogenous ovine-prolactin, bromocriptine or a combination of both treatments. The moult and hair follicle activity were advanced in the prolactin and bromocriptine-plus-prolactin treated groups and delayed in the bromocriptine-treated group, compared to the controls. This conclusivley demonstrated that a rise in plasma prolactin concentration was necessary for the initiation of the moult and hair follicle activity.

The final experiment was designed to determine whether a period of plasma prolactin suppression followed by a rapid increase in plasma prolactin concentration would accelerate the moult and thereby synchronise the onset of moulting within a group of animals. Groups of animals were treated with either bromocriptine, bromocriptine followed by bromocriptine plus prolactin or bromocriptine followed the administration of 'Sulpiride' (a dopamine antagonist and more readily available than prolactin). Consistent with the previous study the onset of moulting was delayed until the end of the period of prolactin suppression. The moult occured rapidly after plasma prolactin elevation and the asynchrony within the groups was reduced slightly compared to the controls.

It is concluded that the timing of the spring moult in the cashmere goat can be altered by manipulating plasma prolactin concentrations. Factors other than prolactin may, however, be involved at the follicular level and controlling the response of individual follicles.

Acknowledgements

I would like to thank the Director of the Macaulay Land Use Research Institute, Professor T.J. Maxwell for the provision of excellent facilities for this project. I would also like to acknowledge the receipt of a Research Studentship award from the Department of Agriculture and Fisheries for Scotland.

I wish to thank my supervisors Dr. A.J.F. Russel and Dr. W.J.M. Black for their support and encouragement throughout the writing of my thesis and for ensuring that all my requirements were met during the experimental periods. Thanks are also due to my Head of Division Dr. J.A. Milne for his support and for constructively reading and commenting on my thesis. My gratitude is expressed to my colleagues at MLURI, and in particular to S. McMillan and Dr. S. Rhind for their excellent technical advice and assistance in radioimmunoassay, to J. Burnett for her invaluable advice on histological techniques and to everyone that held my goats on countless occasions throughout the experimental periods. I would also like to thank D. Elston for his statistical advice, C. Haggerty for Plates I to VI and Figures 3 and 4. Thanks are also due to the sheephouse staff and in particular and to G. Bryce and J. Leary for their assistance with the goats.

I am indebted to Dr. G.A. Lincoln for his endless enthusiasm and encouragement in the developmental stages of the experimental work and for his inspiring discussions throughout this study.

Special thanks are due to my parents for their patience and encouragement and for financially and morally supporting me throughout my many years of education. I also wish to thank all my family and friends for their support and understanding. I would like to express my deepest gratitude to Ivor, my husband, for magnificently adopting the mammoth task of reading my writing, typing my thesis, preparing the diagrams and most of all for maintaining my sanity.

Finally I would like to thank Sandoz, Basle, Switzerland and Sandoz, UK Ltd for their generous gifts of Parlodel (bromocriptine) and to NIDDK, NHPP, University of Maryland School of Medicine, Maryland, USA, for their most appreciated gifts of ovine-prolactin.

CONTENTS

LIST OF TABLES

Chapter Two.

Chapter Three.

Chapter Four

LIST OF FIGURES

Chapter One.

Chapter 2.

Chapter Three.

Chapter Four

Chapter Five

GENERAL INTRODUCTION

'Cashmere goat' is a general name applied to any goat whose winter coat consists of a thick heavy outercoat of long straight, coarse, medullated fibres (also known as beard hairs or guard hairs) and an undercoat of short non-medullated 'down'. Some goats may also have intermediate fibres (gare fibres) with an interrupted medulla (Epstein, 1969).

The zoological origin of cashmere goats has been traced to two species, *Capra falconeri* by Horns (1962), Epstein (1969) and Roberts (1969) and to *Capra hircus blythi* by Kiyathkin (1968). The origin has also been traced to three domestication centres; North-East Kirgizia, South-East Tibet and North-West Mongolia. Due to the considerable variation between goats zoologically classed as cashmere goats, in their quantity of cashmere, body weight and height (Burns *et al,* 1962), it is considered that classification by performance rather than zoological origin may be more appropriate.

Cashmere is defined in biological terms as the fine undercoat of double coated goats, produced by the secondary hair follicles. In the textile industry cashmere is defined as "the fine (dehaired) undercoat fibres produced by a cashmere goat. The fibre is non-medullated and has a mean upper micron limit (diameter) of 18.5 +/- 0.5 microns at a 95% confidence level (projection microscope). The coefficient around the mean should not exceed 24%. There can be no more than 3% of fibres (by weight) over 30 microns (Cashmere and Camelhair Institute of America, 1988).

Cashmere fibre production is a new and developing industry in the UK. The main problem that cashmere producers are faced with, is how to efficiently harvest the cashmere fibre from the goat.

As described above cashmere is the thermal insulating undercoat of the winter coat. In the spring, under the influence of increasing daylength, the cashmere fibres and some of the guard hairs are shed from the hair follicles. The spring

moult takes place over several weeks. There is considerable variation between animals, with moulting occurring as early as February until late May. The cashmere may be harvested from goats by either shearing or combing. Due to the early onset of moulting in some goats, shearing must be completed in February, to prevent any loss of cashmere. However, due to the prevailing climatic conditions in February, housing and hence feeding are necessary to prevent hypothermia and the loss of goats. This adds substantially to the cost of cashmere production. To harvest the cashmere by combing, the goats must be combed during the moult. Due to the length of time required to comb a goat (35 minutes, Rodgers, 1990) and the variation between animals in the timing of the onset of moulting, it is difficult to predict the optimum time to comb a group of animals to minimise the loss of cashmere and to limit the number of times the animals are combed.

Thus it is proposed that by increasing our understanding of the biological control of the moulting process in the cashmere goat, that it may be possible to develop techniques to manipulate the timing and/or the duration of the moult and thereby increase fibre harvesting efficiency. The two approaches considered here are a) to delay the moult so that animals can be shorn without the necessity of a prolonged period of housing, or b) to synchronise and induce the moult in a group of animals so that an optimum time for combing can be more easily selected.

Chapter One: Review of literature

1.0 Introduction

Mammalian skin is covered by a keratinised epidermis which is more pliable than that of reptiles and which gives rise to various epidermal derivatives such as hair follicles, sebaceous glands and sweat glands. Hairs are exclusively a mammalian feature which resulted in the old name for the class *Pilifera* (Johnson, 1977a). The current view is that hairs were completely new epidermal derivatives, although their initial trio arrangement suggests that they originally developed in the interscale hinge region of mammalian-like reptiles, an arrangement which persists on the tails of rodents (Spearman, 1964).

Primitively, hairs may have been primarily tactile and due to the follicles being well supplied with nerves, they can and still do serve this function. However, their main function in present day terrestial mammals is undoubtedly one of insulation (Johnson, 1977a) but hair may also serve as a defence mechanism against abrasion and as camouflage.

The insulative properties of the coat (pelage) are enhanced in many mammals by piloerection which increases the boundary layer of the hair. For the majority of mammals, particularly those that live in a temperate climate where they are exposed to a wide and fluctuating range of environmental temperatures, a seasonally variable insulating layer assists in thermoregulation e.g. the black bear (Johnson, 1977a), the feral goat (Ryder, 1966) and the blue-fox (Smith *et al,* 1987a). As with the breeding season, changes in photoperiod relayed by changes in the duration of melatonin synthesis and secretion, synchronise changes in pelage to seasonal fluctuations in temperature. The association between the timing of the hair cycle and daylength was firstly demonstrated by Bissonette (1935) and has since been demonstrated in many species, including mink (Allain and Rougeot, 1980; Allain, Martinet and Rougeot, 1981), deer (Kay and Ryder, 1978), Soay sheep (Lincoln and Ebling, 1985) and mountain hares

(Flux, 1970).

Goats which have a double coat, comprising of an outer coat of guard hair and an undercoat of cashmere are generally termed 'cashmere' goats. The commercial definition of cashmere is goat undercoat fibre which is less than 18.5 microns in diameter. The breeds of goat normally found in the UK that have cashmere include the dairy breeds, which produce only very small amounts and feral goats which may produce 50 to 100 grammes *per annum*. The Angora goat, the other fibre-producing goat in the UK, differs from the cashmere goat in that it produces only one type of fibre which is known as mohair.

The amount of cashmere present in the coat is dependent generally on the genotype and temporally on the season of the year. In the winter a double coat of cashmere and guard hair is present. The animal undergoes a moult in the spring and a coat consisting of mainly sparse guard hair is maintained over the summer. The cyclical growth and replacement of hair allows for changes in the character of the coat, such as hair density, colour and hair length of thickness (Johnson, 1977b).

1.1 The microscopic structure of skin of the goat (*Capra hircus*).

1.1:1 The skin: The skin, which is the environment of the hair follicles, is comprised of two main layers, a thin outer epidermis and a thicker inner layer known as the dermis or corium (Ryder and Stephenson, 1968). The epidermis of the goat generally consists of four layers from the outermost in, *stratum corneum, stratum lucidum, stratum granulosum* and *stratum ba*sale (Sar *et al*, 1966). The outermost layer of the epidermis, the *stratum corneum*, is made up of flattened dead cells which are continuously sloughed off (Lyne, 1966).

The dermis is much thicker than the epidermis and extends down to the

underlying muscle or *panniculus carnosus* (Lyne, 1966). The layer of the dermis that envelopes the follicles is known as the papillary layer. This is rich in blood vessels and nerves, which together contribute towards the regulation of body temperature. The layer below the papillary layer is the reticular layer, which contains many collagen fibres (Ryder and Stephenson, 1968). Sar *et al* (1966) describe the collagenous fibres of the papillary layer of the dermis as fine, loosely arranged and irregularly distributed, whereas in the reticular layer the collagenous fibres are thick and densely arranged in bundles that lie parallel to the skin surface. No sharp distinction between papillary and reticular layers was evident in most of the body regions of the goat, as the layers blended with each other without demarcation.

1.1:2 The hair follicle group: Hair follicles are termed as being either primary or secondary. Primary follicles are characterised by the presence of three accessory structures: the sebaceous and sudoriferous glands and the erector pili muscle. The secondary follicles are smaller in size, more numerous and have no associated sudoriferous gland or erector pili . The primary follicles are generally arranged in groups of three, termed a trio. The secondary follicles lie between the primary follicles (Ryder and Stephenson, 1968) and together these constitute the follicle group. The mean ratio of secondary to primary follicles in the skin of the goat (Australian cashmere goats) is 6.1:1 (s/p ratio) (Holst, Clarke and Maddocks, 1982), but may vary from 4.1:1 to more than 10.1:1 (Millar, 1986).

1.2 The microscopic structure of the hair follicle of the goat (*Capra hircus*).

1.2:1 The hair follicle: The hair follicle reaches its maximum size and complexity during metanagen, the end of the actively growing phase (anagen). Spearman

(1977b) has given an extensive description of the structures of the hair follicle. The most important points are summarised below. Figure 1 shows the location of each region of the hair follicle.

The hair follicle is a sock-like shape continuous with the epidermis. At the base of the fully developed follicle is the broad bulb which contains the matrix cells. These small round cells divide rapidly during the growth period and are contiguous with the basal cell layer of the upper part of the follicle. At the base of the follicle there is an indentation containing the dermal papilla cells: above the bulb there are the concentric cylindrical layers of the outer and inner root-sheaths which surround the hair fibre and its formative cells. These sheaths and their contained hair are derived from streams of cells proliferated from different regions of the bulb. The root sheaths are formed from the outer part of the bulb, and the hair from the central germinal cells which closely surround the dermal papilla.

At each new cycle of hair growth the region of the follicle between the bulb and the upper limit of the inner root-sheath is completely replaced. The outer root-sheath, the inner root-sheath and the lower part of the hair fibre are referred to as the 'transient' portion of the follicle, as defined by Spearman (1977b) (Figure 2a).

1.2:2 The transient portion of the follicle.

i. The outer root-sheath: This is the thickest cell layer around the hair and is composed of cells very like those of the surface epidermis, except that they do not form either a granular or a horny layer. The upward movement of cells towards the skin surface does not occur to the same extent in the outer root-sheath as in other parts of the follicle. Movement of cells appears to occur towards the inner root-sheath when, in human follicles there is evidence that

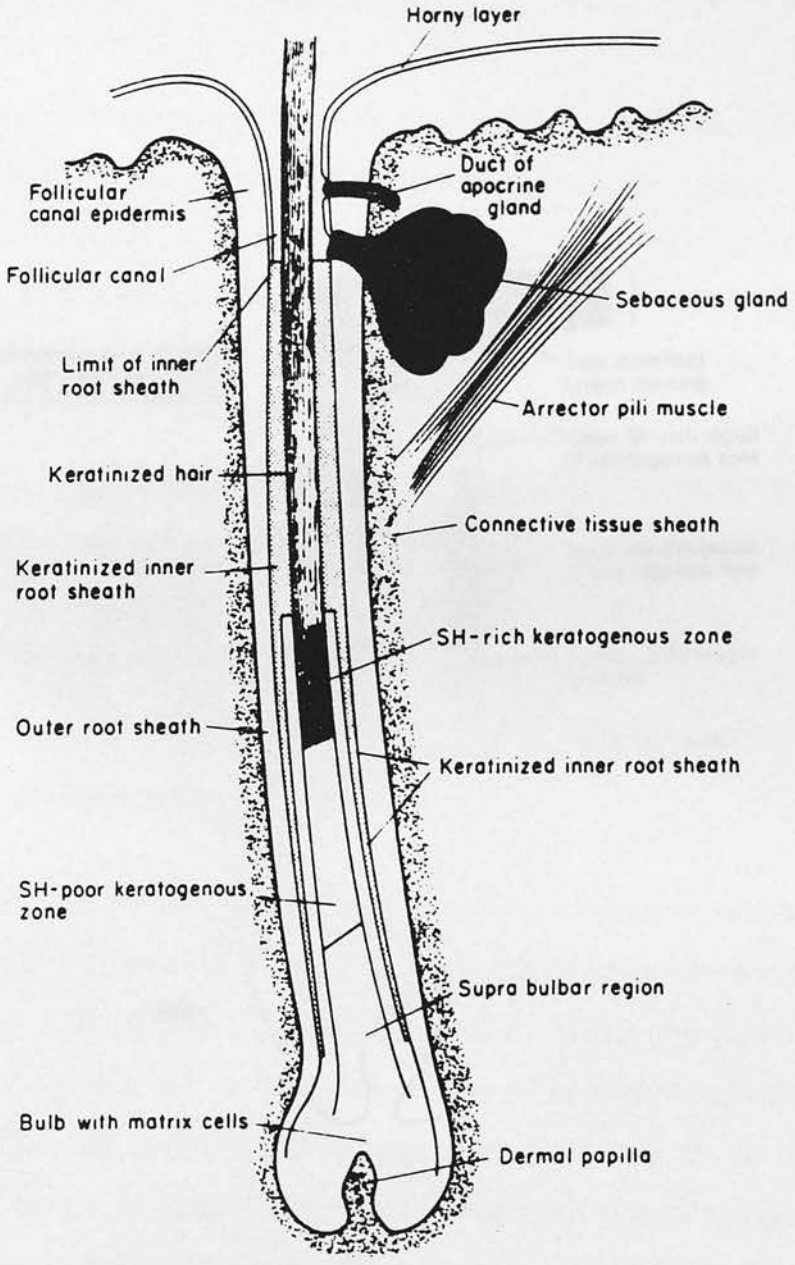


Figure 1. Longitudinal section through a metanagen primary hair follicle showing general topography. (After Spearman, 1977).

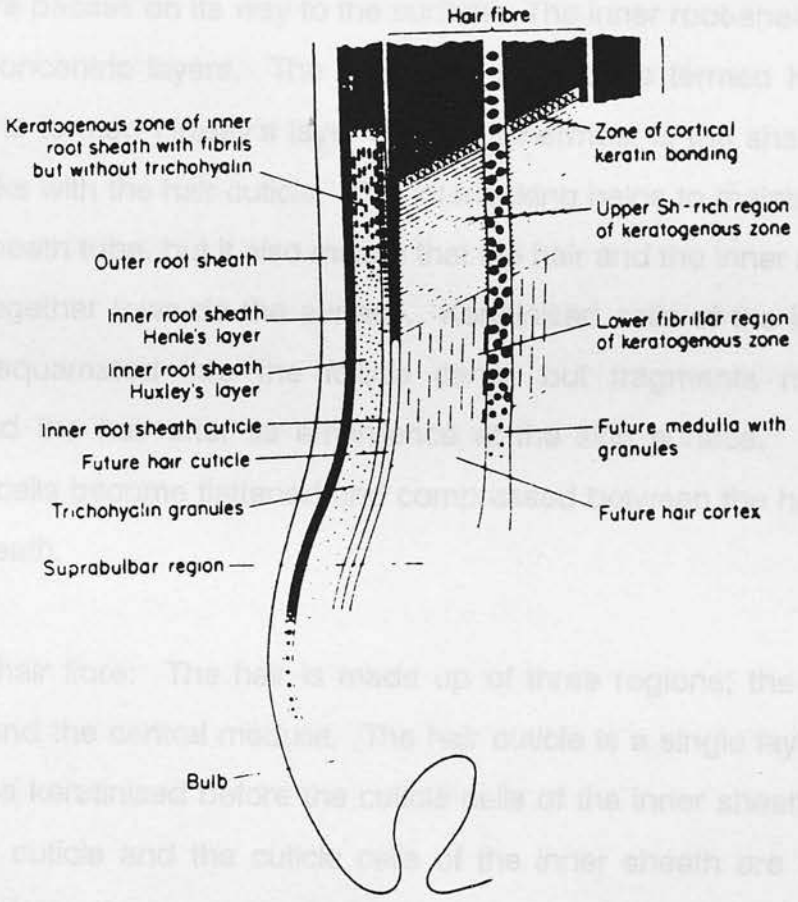


Figure 2a. Diagrammatic logitudinal section through the transient portion of a metanagen follicle showing keratinised regions in grey. (After Spearman, 1977).

some of the cells give rise to the inner root-sheath. In general, it is probable that the inner root-sheath receives contributions of cells from both the bulb and the outer sheath.

ii. The inner root-sheath: The inner root-sheath is much thinner than the outer root-sheath and its keratinised region consists of a rigid tube through which the hair fibre passes on its way to the surface. The inner root-sheath is made up of three concentric layers. The outermost of these is termed Henle's layer, the middle is termed Huxley's layer and the innermost is the sheath cuticle which interlocks with the hair cuticle. This interlocking helps to maintain the hair in the inner sheath tube, but it also means that the hair and the inner root-sheath must grow together towards the surface. Keratinised cells of the inner root-sheath are desquamated into the follicle canal, but fragments may continue to surround the hair after its emergence at the skin surface. The inner root-sheath cells become flattened and compressed between the hair and the outer root-sheath.

iii. The hair fibre: The hair is made up of three regions; the hair cuticle, the cortex and the central medulla. The hair cuticle is a single layer of cells which becomes keratinised before the cuticle cells of the inner sheath. It seems that the hair cuticle and the cuticle cells of the inner sheath are developmentally independent. The cortex is the thickest layer of the hair and is composed of a number of layers of cells. This region keratinises above the zone of the hair cuticle and mechanically it is the strongest part of the fibre. The medulla may be absent in human hairs (Spearman, 1977b) and the majority of secondary goat fibres (Ryder, 1966). The medullary cells are not keratinised and, if present, the cells may shrink and the prominent intercellular spaces fill with

aqueous fluid or air. In secondary fibres the cross section is circular and the cuticular scale pattern of the cornet-like type with both the surface and margins of the scales being smooth (Plate I; Blaze *et al*, 1989). Blaze *et al*, (1989) report that some of the fibres are non-medullated but that they mostly have a central symmetrical type of medulla, which is narrow, unbroken and of the lattice-sponge-like type. The medullar infilling substance is foam-like or of a fibrous or amorphous structure. Ryder (1984) stated that it is the first two secondary follicles which often produce medullated fibres which are coarser than the requirements of the cashmere standard. Goats which produce medullated fibres are not preferred by the cashmere producer and would be selected against in a breeding programme. The diameter of secondary fibres has been reported to range from 8 to 30 microns (Millar, 1986) and 10 to 20 microns (Blaze *et al,* 1989). The differences in specification of secondary fibres between different groups is likely due to the range of goat types which can be classed as cashmere goats.

1.2:3 The permanent portion of the hair follicle.

The permanent portion of the hair follicle is the follicular canal which surrounds the hair, the opening of the duct of the sebaceous gland and the opening of the apocrine gland, in the primary follicle (Figure 2b).

i. The follicular canal: The follicular canal is normally a capillary space between the hair cuticle and the horny layer of the lining of the epidermis. The width of the follicular canal can expand and contract to fit one or more hair fibres. Sebaceous and apocrine fluids lubricate the movement of the fibre past the cell debris formed by the upper portion of the inner root-sheath. The movement of sebaceous and apocrine fluid is by capillarity.

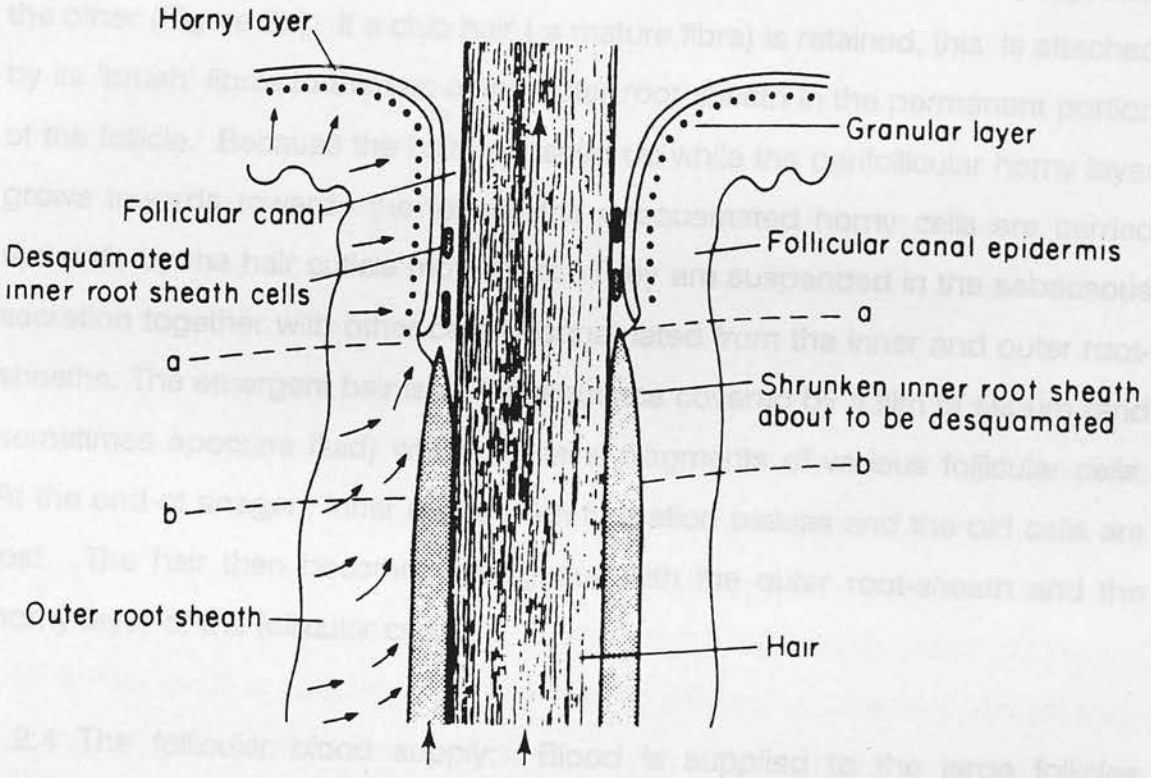


Figure 2b. Section through the upper part of a hair follicle during anagen showing directions of cell movements; a...a separates the outer root-sheath from the epidermis around the follicular canal; b...b is the approximate line between the permanent and transient portions of the follicle. (After Spearman, 1977).

ii. The epidermis: The epidermis of the upper part of the follicular canal is continuous with the lower root-sheath but their functions are quite different. A prominent granular layer is formed by the epidermis of the upper follicle, but keratohyalin granules are not present in the cells of the outer root-sheath. The horny layer is like that of the surrounding epidermis. At the bottom of the follicular canal in the growing hair follicle is the region of desquamation of the inner root-sheath with the hair on one side and the epidermal horny layer on the other (Figure 2b). If a club hair (a mature fibre) is retained, this is attached by its 'brush' fibrils to the sac of the outer root-sheath in the permanent portion of the follicle. Because the hair is pushed up while the perifollicular horny layer grows inwards towards the follicle, the desquamated horny cells are carried upwards by the hair cuticle movement. They are suspended in the sebaceous secretion together with other cells desquamated from the inner and outer root-sheaths. The emergent hair is in consequence covered by a film of sebum (and sometimes apocrine fluid) which contains fragments of various follicular cells. At the end of anagen, inner root-sheath formation ceases and the old cells are lost. The hair then becomes contiguous with the outer root-sheath and the horny layer of the follicular canal.

1.2:4 The follicular blood supply: Blood is supplied to the large follicles (including those of man)by capillary pools which enter the dermal papilla, whereas in smaller follicles a capillary network develops around the base and sides of the follicle (Spearman, 1977b). No work has been published concerning the blood supply of primary and secondary follicles in goats.

2.0 Development of the hair follicle

2.1 Sequence of development in the foetus

Follicular development in foetal skin has been studied in a wide range of species, with most information on sheep (Hardy and Lyne, 1956), laboratory rodents (Hardy, 1951) and humans (Pinkus, 1958). In goats, as in many other mammals, the hair follicles develop when the skin is growing laterally around the enlarging foetus (Spearman, 1977a) and these constitute the adult complement of follicles. In adult mammals under normal conditions there is no postnatal neogenesis of follicles, the only known exception being the annual production of antler velvet in deer (Ebling and Hale, 1970).

Hair follicles develop in a highly ordered sequence. The first follicles to develop are the vibrissae or whisker hairs. Later, follicles develop over the head and body surface and those that appear earliest are the central primary follicles, which are shortly followed by two other primary follicles on either side of the central follicle to form the characteristic trio. These two later forming follicles are known as the lateral primaries. The secondary follicles develop after the primaries. The sequence observed during development (Spearman, 1977a) is the same as the sequence of shedding reported by Ryder (1966) during the hair follicle cycle in dairy goats. The central primary follicles form brush ends prior to shedding before the lateral primary follicles and the two first formed secondary follicles form brushends before the remaining secondary follicles (Johnson, 1977b). It is suggested that the trio group of primaries and their associated secondaries can be considered as a physiological unit with the same sequence of change occurring at any point of the hair cycle.

The timing of primary and secondary hair follicle development in Karakul sheep and dairy goats has been compared by Margolena (1959). The general sequence of events in the development of the skin components and the

primary follicles were very similar in both species, although the initiation and maturation of the secondary fibres in the goat was later. Keratinization of the secondary follicles was close to completion at about 130 days of prenatal life in the sheep whereas in the dairy goats maturation spread from about 135 days of prenatal life to the first postnatal month. In general, the sequence of physiological development of the hair follicles was very similar in the sheep and goat.

2.2 Development of secondary follicles

Development of the secondary follicle population has been monitored in several breeds of sheep by recording the primary to secondary follicle ratio at different stages of pre- and post- natal development (Short, 1955; Burns, 1954). It was reported by Carter (1943) that after birth there was a period of intense secondary follicle activity during which the majority of secondary follicles matured and produced a keratinised fibre. This period of activity was also recorded between birth and 40-80 days of age in the British long-wool and down-wool breeds (Burns, 1954) and from birth to 28-60 days of age in the Australian Merino breed (Schinckel, 1953; Fraser, 1954). Margolena (1959) observed that, in the dairy goat, the first early anlagen of the secondary follicles begins to appear about 3 weeks after the development of the primary follicular generation and that the initiation of secondary follicles was extended through foetal and early postnatal life, however as discussed above keratinization of secondary fibres in the sheep was reported to be completed about 130 days of prenatal life. This is not supported by the work of Ryder (1957) who reported secondary follicle development in Merino lambs several weeks old. It is uncertain whether this difference is due to breed differences or if it is the initiation of new follicles or the maturation of follicles already under development that is

being measured.

Secondary follicles usually arise as separate germs from the epidermis but in sheep the later secondary follicles develop as buds from the necks of earlier secondary follicles. A similar budding from the follicle produces the large compound follicle of the dog (Blackburn, 1965). If budding occurs high in the follicular canal its opening only contains a number of hairs, but if it occurs lower down, a large common follicle is developed having a number of hairs, each with its own inner root sheath.

Developmentally the stimulus to budding is brought about by a division of the dermal papilla which determines hair development. Compound follicles are not always formed by budding from secondary follicles as sometimes a single follicle will divide to form two follicles side by side. Also, occasionally there is incomplete separation of the follicle and its germ, and when this occurs a compound follicle is formed having two parallel medullae.

The role of the compound follicle has been studied by Rougeot *et al* (1984) who suggested that the number of derived hair follicles, formed by branching of particular epidermal follicles, varies according to season and provides additional down (i.e. undercoat) fibres in the winter down of merino sheep and mink.

3.0 The hair follicle cycle.

3.1 Hair replacement: The established hair follicle, throughout the lifetime of the animal, undergoes intermittent periods of growth when a new hair is produced, followed by a period of regression, quiescence and subsequently regeneration of a new fibre (Johnson, 1977b; Spearman, 1977a). This cycle of events is depicted in Figure 3. Cyclical replacement of hairs is necessary for a stable maximum fibre length, to replace worn and broken hair and for any seasonal change in coat colour or density. The maximum length of each fibre

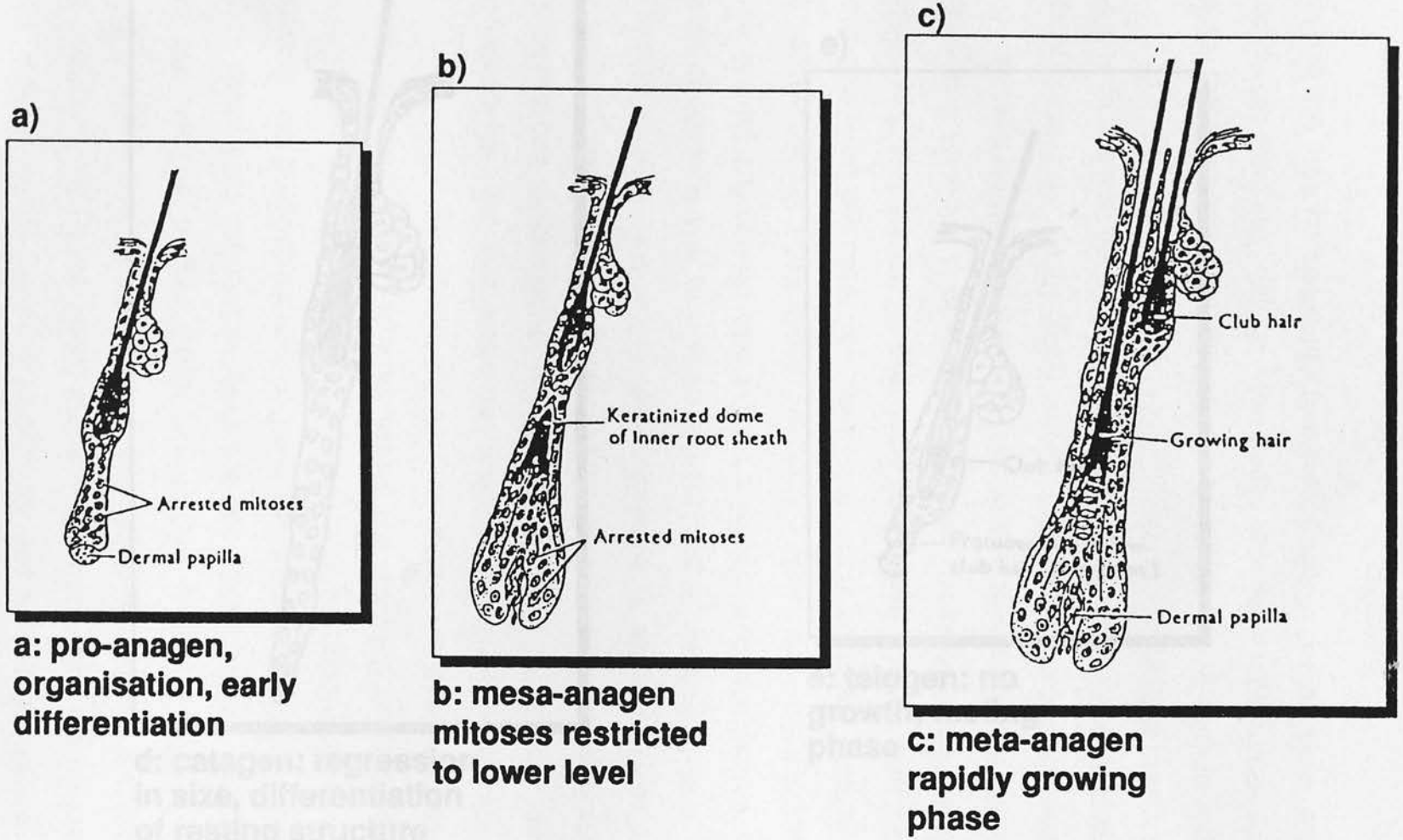
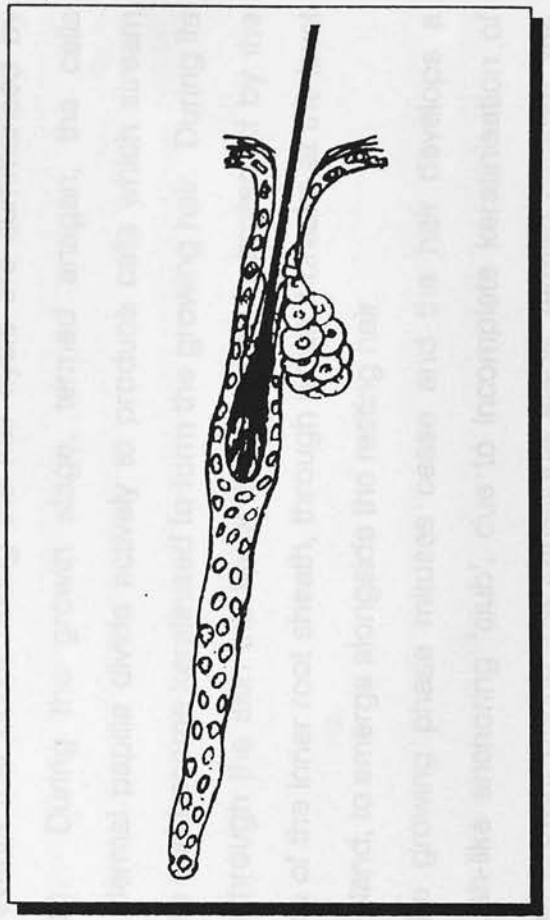


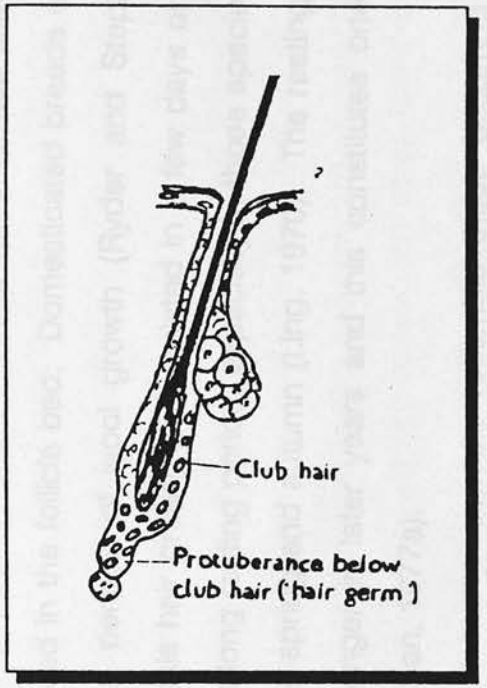
Figure 3. a Cycle of hair follicle growth and replacement, (anagen)

d)



d: catagen: regression in size, differentiation of resting structure

e)



e: telogen: no growth, resting phase

Figure 3. b Cycle of hair follicle growth and replacement, (catagen & telogen)

is genetically determined for each species and is dependent on two factors; namely, rate of hair growth and duration of period of growth (Chase, 1954; Chase and Eaton, 1959; Chase, Rauch and Smith, 1951; Munro, 1956). The most important events that occur during the hair cycle are summarised by Johnson, (1977b). During the growth stage, termed anagen, the cells surrounding the dermal papilla divide actively to produce cells which stream upwards, elongate and become keratinised to form the growing hair. During its growth upwards through the skin the tip of the new hair is protected by the keratininsed dome of the inner root sheath, through which it breaks at the level of the sebacious gland, to emerge alongside the resting hair.

At the end of the growing phase mitoses cease and the hair develops a characteristic brush-like anchoring 'club', due to incomplete keratinisation of cells at the end of growth. During this transitional stage, termed catagen, the follicle migrates upwards to a resting level in the skin, leaving behind an epithelial strand of cells. A subsequent shortening of the epithelial strand occurs by cell degeneration to leave a knob of cells, designated the hair germ, and to expose the dermal papilla. During the resting stage, termed telogen, the club hair is firmly anchored in the follicle bed. Domesticated breeds of sheep have prolonged anagen periods of wool growth (Ryder and Stephenson, 1968), but in most animals hair growth is completed in a few days or weeks, and this is followed by a long resting period, especially in those species which change their coats in the spring and autumn (Ling, 1970). The resting stages become progressively longer in later years and this constitutes one of the factors of ageing (Spearman, 1977a).

The stages of growth in many respects resemble those occurring during follicular development in foetal skin (Spearman, 1977a). The main difference is that the hair cone instead of being formed *in situ* grows up from the germinal

matrix in the bulb. The follicular canal is always patent, and growth changes in the follicular wall can readily alter the diameter of the follicular canal so as to permit the passage of a new hair alongside the old fibre (Chase, 1954).

The stages of the hair cycle can be conveniently described in more detail using the classification system of Chase (1965) which was developed from the terminology and descriptions for the anagen phase of Dry (1926), Chase *et al* (1951), Chase (1958), Hardy and Lyne (1956) and Lyne and Heideman (1959), and for the catagen phase of Straile *et al* (1961).

3.2 Anagen.

3.2:1 Pro-anagen. The morphogenetic phase of growth involves organization of the active follicle with bulb, dermal papilla and early differentiation of inner sheath and hair tip.

Stage I. Changes in the structure of the follicle are related to an increase in size of the dermal papilla cells beneath the germinal matrix of the follicle. The germinal cells at the base of the resting follicle become mitotically very active.

Stage II. The lower portion of the follicle enlarges and grows down to partially surround the dermal papilla. As growth continues the transient portion of the follicle, which atrophied prior to the resting period, is reformed so that the lower portion of the follicle becomes re-established.

Stage III. Maximum follicular length is soon reached and thereafter cellular proliferation results in upward movement of the inner root-sheath and the growing hair fibre. The bulb increases in size and tends to enclose the dermal papilla so that the latter becomes slightly constricted at its lower end. The inactive melanocytes lining the papilla now form dendrites and commence to form melanin granules.

Stage IV. The tip of the new hair grows up from the germinal matrix and this is

surrounded by a concentric cylinder of cells which will form the inner root-sheath. As the tip of the hair approaches the sebaceous canal, the cells become keratinized and at about this level the keratogenous zone of the hair fibre becomes established in the growing hair. The hair makes its way through the desquamating tip of the inner root sheath and reaches the permanent portion of the hair follicle above the sebaceous canal. The keratinized cells of the inner root sheath start to separate, just above the sebaceous canal.

Stage V. The hair is about to enter the follicular canal just above the opening of the sebaceous gland.

3.2:2 Mesanagen. This phase is transitional and very transient. Mitosis becomes restricted to the base of the follicle.

3.2:3 Metanagen. This phase involves rapid growth with no development of new tissues.

Stage VI. The hair emerges from the follicular opening and continues to grow for a predetermined period of time. This is the rapidly growing phase of anagen. Towards the end of metanagen, there is a gradual thinning and lightening of pigment at the base of the hair shafts. The melanocytes in the region of the tip of the dermal papilla cease producing melanin, resorb hair dendrites and become indistinguishable from the matrix cells (Kligman, 1959). At the end of metanagen mitotic activity of the matrix cells gradually slows down and finally stops.

3.3 Catagen: This is another transitional phase in which there is reduction in mitosis at the base of the follicle, the last of the pigmented cells are incorporated into the fibre and the follicle atrophies to about a quarter of its length in anagen

stage VI. Catagen has been primarily considered a degenerative process by Montagna (1956) and Ellis and Moretti (1959) or involving both degenerative loss and dedifferentiation by Kligman (1959). However, Straile *et al,* (1961) proposed that catagen is an orderly sequence of events involving the differentiation of the cells of the hair bulb that remain after the cessation of mitosis. During catagen these cell differentiate to form the last part of the hair, the last part of the internal root sheath and the specialised parts of the telogen follicle i.e. club, capsule, and rounded dermal papilla. Using terminology of Straile *et al* (1961) catagen can be described under the following classification:

Stage I. The production of pigment in the hair bulb stops abruptly and mitoses decline sharply. Morphologically this stage is identical to anagen VI.

Stage II. There is a decrease in the number of pigment-containing melanocytes and bulb cells capping the upper part of the dermal papilla. The dermal papilla is noticeably shortened.

Stage III. The bulb is shorter and narrower than in anagen VI. The dermal papilla is ball shaped and its cells have lost their characteristic spindle shape that they had during anagen. They are more rounded and stain deeply with haemotoxylin, like the cells in the bulb. The last hair cells containing pigment are in the keratogenous zone.

Stage IV. All of the bulb that remains is a narrow column of epithelial cells between the dermal papilla and the keratogenous zone. The last cells that contain pigment are now just above this keratogenous zone and below this is an area of hair which is now non pigmented.

Stage V. The narrow column of epithelial cells between the dermal papilla and the keratogenous zone becomes constricted. This constriction often sets aside a region of bulb cells that cap the dermal papilla. Hair and internal root sheath formation abruptly stop, and a capsule of cells, apparently continuous with the

keratinised internal root sheath, appears around the lower border of the keratogenous zone.

Stage VI. The brush like club differentiates from the cells of the last part of the hair as the hair continues to move upwards. The dermal papilla and many external root sheath cells are left behind. Consequently, the column of epithelial cell between the club and dermal papilla gradually increases in length with the addition of these external root sheath cells. The glassy or vitreous membrane surrounding the column of cells becomes very thick at this time. The keratinised internal root sheath shortens as it moves upwards with the last part of the hair because it continues to slough into the pilary canal just below the orifice of the sebaceous gland. The distance between the differentiating club and the dermal papilla rapidly increases.

Stage VII. The club is fully keratinised and is positioned just below the sebaceous gland. Throughout this stage, the column of epithelial cells between the club and dermal papilla gradually decreases in length. The dermal papilla remains at the end of this shrinking column and, consequently, moves upward. A few cells from the epithelial column and the connective tissue sheath are left trailing behind the ascending dermal papilla with the connective tissue sheath and glassy membrane. The distance between the club and dermal papilla rapidly decreases.

Stage VIII. The dermal papilla has reached the vicinity of the club. The trailing cells disperse, degenerate, or both, leaving only the contracted connective tissue sheath and glassy membrane hanging below the dermal papilla. The continued keratinisation of the hair fibre and the inner root sheath without new cell replacement from the matrix results in a reduction in the size of the hair follicle over the catagen period. The main changes occur over a matter of hours. The last stage of catagen is when the cells at the base of the hair

keratinise to form a brush like extension at the base of the follicle. The keratinised cells become bound to the unkeratinised cells of the outer root sheath of the follicular sac by firm intercellular junctions. The subsequent rupture of these junctions is essential for the final moulting of the club hair from the follicle.

3.4 Telogen: The resting fibre is held within the follicle bed until it is released. The mechanism of moulting is still not properly understood but it must involve the release of the firm junctional complexes between the base of the club hairs and walls of the follicle sac. When the next hair cycle commences the secondary germ elongates by cell division, grows downwards, becomes invaginated by the dermal papilla, and gives rise to new hair bulb. The keratinised dome of a newly formed hair subsequently emerges either by the side of the old club which will be lost at a later time or into the previously established hair canal.

4.0 Patterns of hair growth and development

Many mammals have a seasonal pattern of pelage replacement which demonstrates that there must be a certain degree of synchronisation between follicles over the body surface enabling the animal to undergo seasonally regulated changes in coat colour or density. The most common type of hair replacement involves the initiation of follicular activity in a specific region of the skin in synchrony with neighbouring follicles. The area of activity expands in a characteristic pattern so that it then passes like a wave over the enter skin.

In domesticated mammals, such as the mouse and rat, the coat cycle has become disengaged from any environmental influence. The laboratory rat exhibits several coat changes which occur at various stages of maturity. Hair

growth starts ventrally and proceeds as a wave across the flanks to the dorsum and thence to the head and tail, demonstrating synchrony between groups of hair follicles (Johnson, 1958). Similar patterns of hair replacement have been described for the mouse (Dry, 1926) Mouflon (Ryder, 1960) and Wiltshire Horn sheep (Slee, 1959). The gradual change in hair replacement demonstrated in these species has obvious advantages in preventing any sudden alterations in thermal insulation of the pelage (Johnson, 1977b). In man the activity of each follicle is independent of its neighbours and may possibly represent a further stage of domestication.

In sheep there is wide variation between breeds in their tendency to shed fibres, from a complete spring moult in the primitive Shetland and Soay sheep to almost continuous wool growth in the Merino (Panaretto, 1979). Sheep genotypes which shed their fleece annually include the Wiltshire Horn (Slee, 1965; Ryder, 1969), the Soay (Ryder, 1971) and the Mouflon (Ryder, 1973). Kemp hairs, which are the coarsest of the primary guard hairs, are shed annually in the Limousine (Rougeot, 1961).

In breeds of sheep such as the Merino or Cheviot, wool fibres continue to grow for more than one year and the shedding of one fibre is independent and therefore not synchronised with neighbouring follicles. However, seasonal shedding of hair from the legs of Merino sheep has been described by Hutchinson (1965).

Seasonal changes in coat structure in goats were first detailed in a study by Ryder (1966). Monthly coat and skin samples were taken from three goats (Saanen and Saanen x Angora) for one year in Australia and from six others (Saanen and Toggenburg) for two years in Scotland. The coat appeared to have a simple cycle of active growth in summer and inactivity in winter. The kemp fibres of the outer coat formed brush ends at about the time of the

autumn equinox and remained dormant until late spring. Kemp shedding in the skin demonstrated a pattern of maximum activity in late summer with almost all kemp fibres forming brush ends during winter. There was little or no difference between individuals, although there was tendency for regrowth earlier in males than females. It should be noted that in the manufacturing industry kemp fibres are a type of fibre characterised by a very coarse and chalky appearance quite distinct from guard hairs and that in this paper the author refers to all fibres produced from primary hair follicles as kemp fibres.

McDonald (1985) described the annual cycle of the fibre length of Australian cashmere goats under natural photoperiod. Maximum length was achieved at the winter solstice and minimum length of cashmere at the summer solstice, representing an absence of cashmere from the coat during the summer. A similar pattern emerged for the proportion of cashmere in the coat with maxima at the winter solstice and minima at the summer solstice. This closely resembles the cycle of hair follicle activity previously described by Ryder (1966) with high activity and therefore rapid growth occurring from the summer to the winter solstices, resulting in maximum cashmere length in winter. In a more recent study McDonald *et al* (1987) followed annual cycles of length, diameter, volume growth rate (VGR), brush end formation and cashmere to hair ratio (CHR; a measure of the proportion of cashmere present in the coat) of Australian feral goats. This work was reported from the Southern Hemisphere and for clarity the Northern Hemisphere month equivalents are given in parentheses. Cycles of cumulative length for both cashmere and hair were synchronous with maxima occurring in June (December) and July (January) respectively.

The cycle of cashmere diameter had minima occurring in June/July (December/January) and February (August). The annual cycle of VGR of cashmere and hair was asynchronous with maxima for cashmere in April

(October) and for hair November (May). Indeed the maximum VGR of hair was achieved when cashmere was in telogen. It was hypothesised by McDonald *et al (1987)* that as the changes in VGR, which comprises both length and diameter of the fibre are asynchronous between cashmere and hair, the mechanisms controlling the rate of follicle activity are specific to primary and secondary hair follicles. VGR illustrates the composite effect of independent cycles of the two components and therefore if cycles of length are synchronised it can be assumed that differences in volume growth rate are due to differences in diameter of the fibres produced.

Oliver (1969) previously demonstrated that the diameter of a fibre produced by an individual hair follicle is regulated by the size of the dermal papilla, thus indicating that follicle specific mechanisms controlling dermal papilla size rather than rate of follicle activity may be involved.

As changes in length of cashmere and hair are synchronised this suggests a common mechanism controls the rhythmic activity of primary and secondary hair follicles. The reduced amplitude of the cycle of hair length compared to cashmere length means that goats maintain a protective hair coat when little or no cashmere is present.

The annual cycle of brush end formation of both primary and secondary follicles was associated with the cessation of growth in December/January (June/July) and a subsidiary event between December (June) and March (September). The maximum cashmere to hair ratio achieved (5.9:1) did not reach the skin s/p potential (6.9:1), indicating that there was irreversible loss of cashmere fibres following either subsidiary cycles or perhaps as previously discussed, due to the inefficient development of secondary follicles after birth (Burns, 1954).

The absence of secondary follicle fibres over the summer months in the

Mouflon has been explained by the presence of subsidiary cycles of secondary follicle activity. At no point in the annual cycle was secondary follicle activity 100%, indicating that different follicles were perhaps included in successive samples and that secondary follicle moulting was occurring in waves (Ryder, 1966). The presence of subsidiary cycles of secondary follicle activity in cashmere goats has been demonstrated by Nixon *et al* (1991), however the results were interpreted as indicating the growth of vellus fibres, which are extremely small fibres almost invisible to the naked eye, which moult at the end of the summer when the true cashmere fibres begin to grow to form the winter coat. No other observations of vellus fibres have been reported.

Ebling and Hale (1970) suggest that fibres which have formed brush ends are not shed from the follicle until a new hair commences growing. This mechanism is not supported by (McDonald *et al*, 1987) who report no evidence of follicle activity when fibre shedding is taking place as cashmere VGR is zero when the CHR ratio is declining, indicating a pronounced period of telogen in cashmere-bearing secondary follicles following fibre shedding. There is no obvious mechanism by which the fibre is lost from the follicle although it must involve breaking of the bonds between the brush end and the follicle and also synchronisation between the secondary follicles.

5.0 The control of hair growth.

5.1 The control of hair type.

When hair is transplanted between regions of the body the hair produced is always characteristic of the follicle and not the recipient site (Johnson, 1977b) for instance, guinea-pig hair shows persistence of both length and colour pattern when transplanted (Seevers and Spencer, 1932) and similarly, when

hair is transplanted from a haired to a hairless mouse, hair continues to grow on the transplant (Argyris and Argyris, 1970). The determinants of hair characteristics remain unsolved (Johnson, 1977b) although interactions between the epidermal and dermal components appears to be involved. Oliver and Jahoda (1989) have clearly demonstrated by tissue culture techniques that the dermal papilla component of the follicle is a prerequisite for successful regeneration of follicle. It has also been suggested that in adult hair follicles the size of the dermal papilla determines the size of the population of germinative cells in the matrix and hence the size of the hair produced (Van Scott *et al*, 1963). Indeed in adult hair follicles the size of the dermal papilla is correlated with the size of hair or wool fibre produced (Burns and Clarkson, 1950; Straile, 1965) and when the hair produced from a particular follicle changes in size, as happens in humans when axillary, pubic and beard hairs enlarge at puberty or scalp hairs diminish in male-pattern baldness, there is a concomitant change in the size of the dermal papilla (Johnson, 1977b).

5.2 The initiation of hair growth.

The main issue concerning hair growth is the physiological mechanism responsible for the initiation of each cycle.

There are several levels of control of an individual hair follicle which regulate the characteristics of the hair produced and the timing of each component of the hair cycle. The controlling factors may be inherent within the follicle, under systemic control or modified by the relationship between systemic, local and inherent factors. In many mammals, waves of activity pass over the body in definite patterns. In an attempt to explain these wave patterns Mercer (1961) proposed that nearby follicles may affect one another, as an inhibitor accumulates or disperses, so that activity was propagated from one row of

follicles to the next. The role of an inhibitor within the follicle was previously advanced by Chase (1954) from the evidence that a quiescent follicle was always brought into activity by plucking. He suggested that an inhibitor accumulates within the follicle during active growth and that when it reaches a critical concentration growth ceases. During the resting stage the inhibitor breaks down or is dispersed and if the hair is plucked from the follicle the inhibitor is removed along with the club hair. This theory was tested in the laboratory rat by Johnson and Ebling (1964). When the hairs were plucked at progressively later times during the resting period, between 20 and 12 days before expected eruption, hairs consistently erupted 11 days after plucking. Thus the time by which the activity was advanced decreased progressively until at 12 before eruption plucking had no effect. When hairs in resting follicles were plucked at between 9 and 6 days before their expected eruption, the interval between plucking and eruption remained the same and consequently delayed activity compared to the controls.

When hair was plucked from the follicle during the active phase, the active phase of the follicle was shortened and the length of the following resting phase remained consistent with that of the controls. This contradicts Chase's (1954) theory of an inhibitor accumulating during the active phase which is dispersed during telogen. If this was true, plucking during activity would result in a decrease in the resting phase due to a lesser build up of inhibitors rather than a reduction in anagen and no change in telogen.

When a follicle has been plucked the replacement hair continues out of phase with surrounding hairs (Chase, 1954) indicating that the follicle has a persistent intrinsic rhythm. Ebling and Johnson (1961) rotated homografts 180 degrees to the rest of the skin and the wave of activity passed dorsal to ventral compared to ventral to dorsal on the rest of the body. The mean lengths of the

hairs and the order of activity were consistent with their origin. This is not consistent with Mercers (1961) theory of propagation. Evidence of an intrinsic rhythm within follicles is also demonstrated in experiments involving the transplantation of follicles between animals of different ages (Ebling and Johnson, 1961). When skin was transplanted from a 28 day old to a 49 day old rat, eruption of hair occurred at the same time on the transplant as on the donors autograft, in both the first and second waves of hair growth after grafting, i.e. asynchronous with the recipient. This suggests an inherent rhythm in follicular activity is present, which can persist for at least two cycles irrespective of systemic factors. Paradoxically, when skin is transplanted from the 49 day old rat to the 28 day old rat, the opposite effect was obtained. Growth of hair was stimulated in the grafted area, at the same time as for the recipient. This indicates that systemic factors can also be involved in synchronising one area on the flank with the surrounding area. In conclusion, this suggests that both inherent and systemic factors are involved in controlling the hair follicle cycle and that inherent rhythms may be paramount in the 28 day old rat, whereas systemic factors may override inherent factors in the older animal.

6.0 The endocrine control of hair growth and moulting.

Mammals such as laboratory rats, exhibit waves of growth which correspond to a specific age. In contrast, mammals which require thermoregulatory changes in the insulative quality of their pelage to survive seasonal changes in temperature, exhibit annual cycles of hair growth and replacement. As early as 1935, Bissonette demonstrated that the timing of the moult in ferrets was related to daylength. Since then, many findings have confirmed that photoperiodic changes are the main proximate factor, synchronising seasonal

cycles in reproduction, hair growth and appetite to optimise an animal's chances of survival for itself and its offspring (see Arendt, 1986; Lincoln, 1986; Martinet and Allain, 1985; for reviews).

6.1 Melatonin.

Melatonin is an indoleamine, synthesised and secreted by the pineal gland (*epiphysis cerebri*) in response to the dark phase of the circadian photoperiod. Melatonin is synthesised from a second pineal indoleamine, serotonin, through the action of two enzymes; N-acetyltransferase (NAT), which is responsible for the N-acetylation of serotonin, and hydroxyindole O-methyltransferase (HIOMT) which is responsible for the O-methylation of the indole ring (Tamarkin *et al*, 1985; Klein *et al*, 1981). Quay (1963, 1964) observed that levels of serotonin are high during the day and low at night, and more importantly that changes in the lighting cycle caused corresponding changes in pineal serotonin content. He also demonstrated a daily rhythm in pineal melatonin which was the inverse of the serotonin rhythm. In a review of melatonin production Tamarkin *et al* (1985) suggest that the nocturnal rise in NAT activity may be responsible for driving the melatonin rhythm, whereas HIOMT activity may determine, at least in part, the amplitude of the nocturnal increase in melatonin. Concentrations of melatonin have been found to increase at night in the pineal gland, blood, cerebrospinal fluid and urine in all mammalian species studied to date (Klein *et al*, 1981).

Photic information reaches the pineal through a pathway involving the eye, the retino-hypothalamic tract and sympathetic innervation from the superior cervical ganglion (Lincoln, 1984). More specifically, nerve impulses stimulated by light impinging on the eyes are transmitted via a retino-hypothalamic tract to the hypothalamic suprachiasmatic nuclei (SCN), which function as

autonomous, central, circadian oscillators (Rusak and Zucker, 1979) and then to the paraventricular nuclei (Klein *et al,* 1983). From these hypothalamic nuclei the impulses traverse fibres in the medial forebrain bundle and reticular formation to the intermediolateral nucleus of the spinal cord. From there they pass to preganglionic adrenergic fibres of the sympathetic nervous system which conduct them to the superior cervical ganglion (SCG). The final sympathetic input to the pineal arises from the SCG (Tamarkin *et al,* 1985).

As discussed above, melatonin is produced in a circadian pattern with peak plasma concentrations being attained during the hours of darkness and basal levels occurring during daylight. Thus the daily pattern of melatonin secretion i.e. the duration of melatonin elevation, varies with season and is inversely related to daylength. The difference in duration of plasma melatonin concentration is clearly illustrated in Figure 4.

Experiments, using pinealectomised or superior cervical ganglionectomised animals, have demonstrated the role of the pineal gland in coordinating melatonin production to changes in photoperiod rather than as a rhythm generator. For example, Martinet *et al* (1985) studied the effect of pineal denervation, which precludes melatonin synthesis, on the timing of changes in live weight, plasma prolactin concentrations and moulting periods in mink. Under natural photoperiod, for the duration of the experiment, no differences were observed between the control and pinealectomised animals, suggesting an inherent rhythm controlling the observed physiological events that is not dependent on melatonin synthesis. However, when challenged with an artificial change in photoperiod, the control animals responded to a change to long daylength with an increase in plasma prolactin concentration and onset of the spring moult whilst a change to a short daylength resulted in a decrease in plasma prolactin concentration and onset of the autumn moult. The pineal

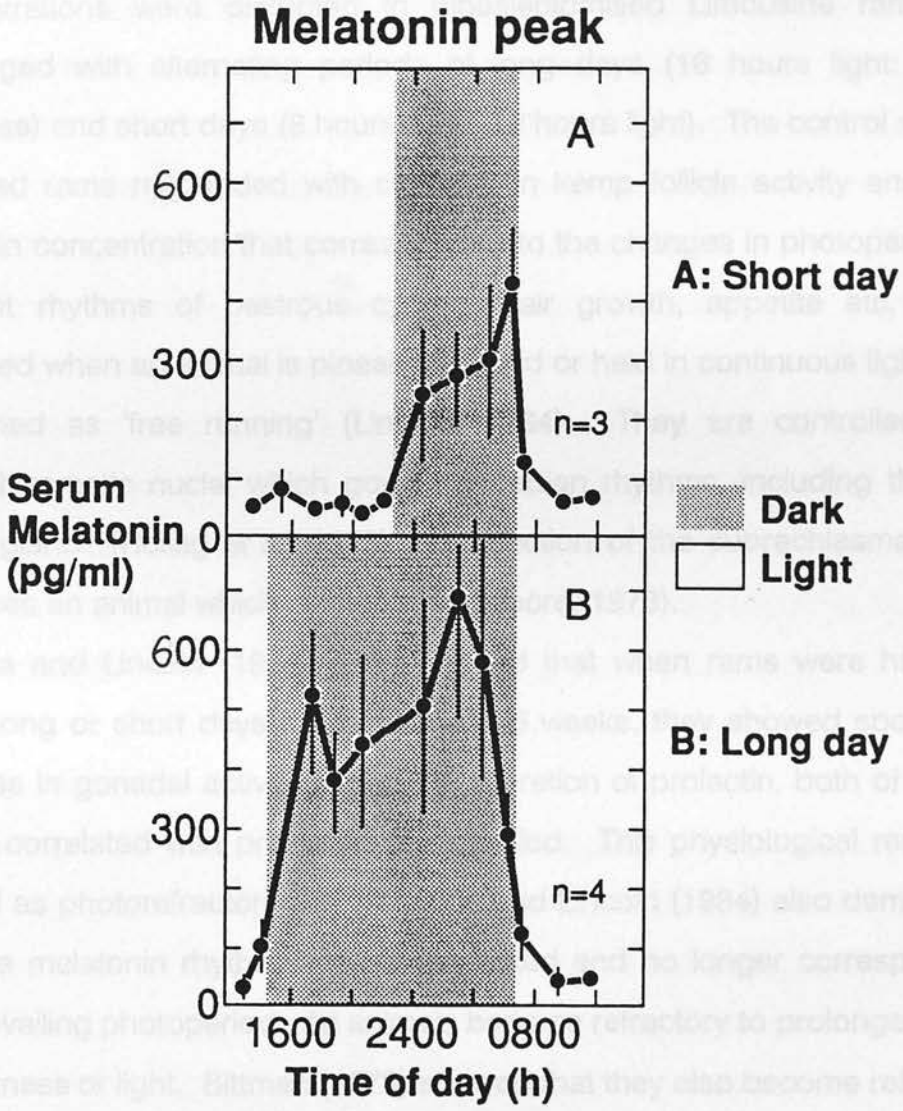


Figure 4. The difference between short and long days in the duration of the rise in plasma melatonin concentration. (after Arendt, 1986.)

denerved animals exhibited no such changes indicating that without a circadian pattern of melatonin synthesis they could not respond to a change in the photoperiod. This is in accordance with the findings of Allain *et al* (1986) who demonstrated that the kemp hair follicle cycle and plasma prolactin concentrations were disrupted in pinealectomised Limousine rams, when challenged with alternating periods of long days (16 hours light: 8 hours darkness) and short days (8 hours dark: 16 hours light). The control and sham operated rams responded with changes in kemp follicle activity and plasma prolactin concentration that corresponded to the changes in photoperiod. The inherent rhythms of oestrous cycling, hair growth, appetite etc. that are observed when an animal is pinealectomised or held in continuous light or dark is termed as 'free running' (Lincoln, 1984). They are controlled by the suprachiasmatic nuclei which govern circadian rhythms, including that of the pineal gland (Rollag *et al,* 1978). Destruction of the suprachiasmatic nuclei produces an animal which is arhythmic (Moore, 1978).

Almeida and Lincoln (1984) demonstrated that when rams were held under either long or short days for more than 16 weeks, they showed spontaneous changes in gonadal activity and in the secretion of prolactin, both of which no longer correlated with prevailing photoperiod. This physiological response is termed as photorefractoriness. Almeida and Lincoln (1984) also demonstrated that the melatonin rhythm became disrupted and no longer corresponded to the prevailing photoperiod. As animals become refractory to prolonged periods of darkness or light, Bittman (1978) showed that they also become refractory to prolonged melatonin administration.

6.1:1 Manipulation of the hair follicle cycle by melatonin administration.

Melatonin implants have been used to manipulate the timing of the coat cycle in

mink (Allain et al, 1981; Martinet and Allain, 1985). When melatonin, given in the form of a slow release implant, was administered in July, the autumn moult occurred 4 weeks later and the development of the winter coat was advanced by about 2 months compared to the controls. This was accompanied by an increase in plasma prolactin concentration. Testicular recrudescence began when the moult was complete. When melatonin implants were given in January or March before the Spring moult, the normal decrease in body weight and testes volume, the increase in plasma prolactin concentration and the onset of the spring moult were all delayed by 3 months.

In the male Blue Fox, melatonin administration for one year from August, resulted in normal redevelopment of the testes and growth of the winter coat during autumn, but prevented testicular regression, the moult to a summer coat the following spring and the spring rise in plasma prolactin concentrations (Smith *et al*, 1987a). These results emphasise that melatonin treatment can alter the timing of seasonal events , including hair growth and moulting, but that the response obtained is dependent on the timing of the treatment.

6.2 Prolactin

The hormone prolactin is synthesised and released by the lactotroph cells of the pituitary gland. Annual cycles of prolactin with peak levels in the summer and basal levels in winter have been demonstrated in many species, including sheep (Ravault, 1976), male goats (Buttle, 1974), rats (Amenori *et al*, 1970) and Blue Foxes (Smith *et al* (1987b). These seasonal cycles can be modified by photoperiod (Pelletier, 1973; Lincoln *et al,* 1978). Prolactin has been implicated in many physiological events, including seasonal pelage changes (Duncan and Goldman, 1984; Smith *et al*, 1987), growth (Eisemann *et al,* 1984) and lactation (Forsyth, 1983).

The hypothalamic inhibition of prolactin secretion and synthesis has been reviewed by Ben-Jonathan (1980). Hypothalamic lesions or disconnection of the pituitary from the brain, either by stalk section (Diefenbach *et al*, 1976; Kanematsu and Sawyer, 1973) or by transplantation of the pituitaries to other sites in the body (Everett, 1954) results in the cessation of secretion of all trophic hormones while that of prolactin increases. The reverse occurs when hypothalamic extracts are added to incubated pituitaries (Talwalker, Ratner and Meites, 1963), resulting in a dose-related increase in the secretion of LH, FSH and TSH, concomitant with a proportional reduction in the release of prolactin. Orstead and Blask (1987) reported that the inhibitory control of the hypothalamus on prolactin synthesis and secretion is achieved via the action of prolactin inhibitory factors or hormones released in to the hypophyseal portal vasculature. They demonstrated that dopamine, which inhibits pituitary prolactin cell activity by binding to specific dopamine receptors (Ben-Jonathan, 1985) led to a substantial inhibition (70-80%) of the release of prolactin from male hamster pituitaries *in vitro*. However, prolactin synthesis remained unaffected. It was concluded that dopamine may be an important regulator of short term prolactin release but not synthesis, in the long photoperiod exposed male hamster. In addition Blask *et al* (1986) have demonstrated that long-term light deprivation inhibits the release, storage and synthesis of prolactin in both male and female Syrian hamsters. This suggests that pineal-mediated prolactin suppression involves one or more prolactin-inhibitory factors other than dopamine. Indeed Dannies (1982) comments that the release of prolactin is not a simply regulated process, but is a complex series of events which include synthesis, storage, release and degradation.

6.2:1 Manipulation of the hair follicle cycle by prolactin manipulation.

A relationship between changes in plasma prolactin concentration in response to changes in daylength and kemp follicle activity was demonstrated by Allain *et al* (1986). When plasma prolactin concentration was acyclic in pinealectomised animals, kemp follicle activity oscillated around 50% (of the follicles tested) which is indicative of asynchrony in the hair follicle cycle. Changes in the timing of the moult to summer pelage, and in the Djungarian hamster to winter pelage in the autumn, have been achieved by manipulating plasma prolactin concentrations. In the male Blue Fox, plasma prolactin suppression by slow release injections of bromocriptine (a dopamine agonist) from March to May resulted in a delay in the spring moult. The animals retained winter coats of varied quality and maturity until the end of the study (Smith *et al*, 1987b). Curlewis *et al* (1988) achieved partial suppression of plasma prolactin concentration in red deer hinds by the administration of bromocriptine, from mid-winter. The onset of anoestrus, the seasonal reduction in VFI and the moult from winter coat to summer coat were all delayed, demonstrating that changes in plasma prolactin concentrations are not solely associated with hair cycles. Effects of prolactin on appetite and reproduction also require to be considered in experiments, when hair growth is being investigated. A delay in the spring moult to pigmented hair in the Djungarian hamster, as a result of bromocriptine administration, was overcome by concomitant prolactin injections (Duncan and Goldman, 1984). Niclowitz and Hoffman (1988) also demonstrated an association between plasma prolactin concentration and the autumn moult to white winter pelage in the Djungarian hamster. Hypophysectomised hamsters maintained under a long photoperiod exhibit an autumn moult to the white coat normally obtained in the autumn under the influence of decreasing daylength. The change to winter pelage was prevented by prolactin administration, indicating that suppression of

plasma prolactin is necessary for the acquisition of winter pelage.

The change to dark pigmentation in weasels was previously attributed to melanocyte stimulating hormone (MSH) and adrenocorticotrophic hormone (ACTH) by Rust (1965). Hypophysectomised weasels also undergo an irregular and asynchronous moult to white winter pelage under any photoperiodic influence. Rust (1965) demonstrated that dark pigmentation could be restored by treatment with MSH and ACTH. Rust and Meyer (1968) also demonstrated that ectopic pituitary autografts had the same effect and thereby concluded that these contained MSH. In a more recent experiment, Duncan (1980) transplanted ectopic pituitary autografts to Djungarian hamsters exposed to short photoperiod. This similarly resulted in mainte\nence of the dark pelage. In addition, however high circulating levels of prolactin were recorded. Niclowitz and Hoffman (1986) conducted an experiment to demonstrate conclusively the role of prolactin and/or MSH and ACTH in restoring pigmentation. Hypophysectomized hamsters were treated with prolactin, MSH or ACTH. Only those receiving prolactin moulted to the dark summer pelage. It is concluded that, at least in this species, high prolactin concentrations are necessary to initiate hair follicle acitivity and the spring moult and to convey the signal from the pituitary to the melanocytes.

6.3 Thyroid hormones

The thyroid hormones, triidothyronine (T3) and thyroxine (T4), are produced by the thyroid and regulated by the pituitary glycoprotein gland's thyroid stimulating hormone (TSH) (Vriend, 1983).

The control system regulating thyroid hormone secretion is usually described as having three major components, the thyroid gland, the pituitary and the hypothalamus. Stores of thyroid hormones in the form of thyroglobulin in the

thyroid follicles and in the form of T4 and T3 bound to plasma binding proteins ensure an adequate supply of free hormone available for uptake and utilization. In physiological conditions of thyroid hormone excess, feedback inhibition of TSH secretion by T4 occurs and in the case of thyroid hormone deficiency an increase in TSH secretion occurs (Vriend, 1983).

A reduction in wool growth rate has been reported in sheep after thyroidectomy. In sheep maintained on constant feed intake, thyroidectomy depressed wool growth to approximately 60% of the preoperative rate (Thierez and Rougeot, 1962; Ferguson *et al,* 1965) The reduction in wool growth appeared to be entirely due to a reduction in fibre length, fibre diameter being unchanged (Theriez and Rougeot, 1962). Wool growth rate was restored to preoperataive rates by subcutaneous injections of T4 (Ferguson *et al*, 1965). In addition, by increasing the dose of T4, wool growth rates could be increased above preoperative rates (Ferguson *et al* , 1965). Indeed, wool growth rate can be increased in normal sheep by treatment with T4 (Labban, 1957; Ferguson, 1958). However in hypophysectomised sheep, T4 injections can increase wool growth to preoperative rates but not greater (Ferguson *et al,* 1965). This implies that some pituitary factor is necessary for an increase in thyroid hormone treatment to express its full physiological potential.

Thyroid hormones display a seasonal pattern of plasma concentration (Wallace, 1979 a and b) but these do not correlate with changes in rate of wool growth. It is concluded that thyroid hormones are necessary for an animal to reach its potential in hair growth, seasonal changes in T4 or T3 are not however, necessarily associated with seasonal coat growth and moulting.

7.0 Temperature

Although photoperiod appears to be the proximate factor controlling seasonal

changes in pelage, there is evidence of a modifying effect of temperature (Johnson, 1977a and b). Weasels (*Mustela*) kept on long days at warm or cold temperatures all changed to a dark, summer pelage (Rust, 1962). However the change to brown pelage occurred more slowly in the cold temperature housed animals due to a delay in the shedding of the white winter coat. The Scottish mountain hare *(Lepus timidus scotias*) also exhibits a change from white winter pelage to brown summer pelage and *vice versa*, but the rate of the progression of the moult in either direction appears to be dependent on temperature (Hewson, 1958; Watson, 1963 and Flux, 1970).

8.0 Appetite

In both sheep and deer a seasonal depression in voluntary food intake (VFI) has been observed, the nadir of the annual cycle occurs in winter (Curlewis *et al*, 1988, 1991). Diurnal and circannual cycles of appetite in sheep have been partially associated with changes in melatonin secretion (Schanbacher and Crouse, 1981), ie. sheep ate less frequently and smaller amounts during the hours of darkness, when melatonin production is at its peak. Deer have a very pronounced winter depression in VFI, linked to the decrease in photoperiod experienced at this time (Curlewis *et al,* 1991). Manipulation of the circannual plasma prolactin concentration cycle results in associated changes in VFI. This demonstrates that manipulation of photoperiodic hormones, such as melatonin and prolactin, can have profound effects on many physiological functions and not simply those observed or challenged.

Conclusions.

1. Hair follicles have an inherent rhythm which dictates the pattern of moulting over the body surface.

2. The inherent rhythm of the hair folicle is entrained by photoperiodic changes to an annual cycle of hair growth and replacement.

3. Changes in photoperiod are communicated through the eye to the pineal gland, via the suprachiasmatic nuclei and the superior cervical ganglion. The brain perceives changes in photoperiod by the associated change in the duration of melatonin synthesis and secretion

4. Melatonin administration can be used to manipulate coat growth and moulting, the response, however depends on the timing of the treatment in relation to the hair follicle cycle.

5. Melatonin may possibly exert its effect on hair follicle activity via the hormone prolactin.

6. Manipulation of plasma prolactin concentration alters the timing of the moult.

7. Circannual changes in T3 and T4 do not correspond to changes in the hair follicle cycle.

8. Manipulation of seasonal cycles of hair growth and moulting may result in corresponding changes in VFI and reproduction.

Chapter Two: The effect of exogenous melatonin treatment from December until May on plasma prolactin, thyroxine and triiodothyronine concentrations, appetite, live weight, fibre shedding and hair follicle activity.

Experiment One: The effect of exogenous melatonin treatment from December until May on plasma prolactin, thyroxine and triiodothyronine concentrations, appetite, live weight, fibre shedding and hair follicle activity.

AIM

The aim of this experiment was to investigate the possibility of altering the timing of the Spring moult in the Cashmere goat by the use of exogenous melatonin.

INTRODUCTION

In the cashmere goat, the spring moult from the winter to the summer coat may start as early as February. If the cashmere is to be harvested by shearing, the goats have to be shorn in early February before any cashmere has been shed from the coat, to maximise harvesting efficiency. Due to the time of shearing relative to the British climate, cashmere goats have to be housed after shearing to prevent hypothermia. A delay in the moult until May would considerably reduce the period of housing and supplementary feeding required, thereby increasing the economic efficiency of cashmere production.

The role of the pineal gland, and its product melatonin, in regulating the spring and autumn moults in the mink has been well established (Martinet and Allain, 1985). Silastic capsules of melatonin implanted in July advanced the autumn moult and development of the winter coat by two months. The moult was accompanied by a decline in plasma prolactin levels and an increase in body weight. When capsules were implanted in January or March, the spring moult to a summer pelage, the seasonal decrease in body weight and testes volume and the normally associated rise in prolactin levels, were all delayed by three months (Allain *et al*, 1981).

A delay in the spring moult following melatonin treatment has also been

demonstrated in the male blue-fox (Smith *et al,* 1987a). Melatonin implants were administered for one year from August, there was normal establishment of the winter pelage and development of the testes in the autumn, but the rise in plasma prolactin concentration, testes regression and the moult to a sum mer coat which normally occur in the spring, were all delayed until the end of treatment.

In this study the possibility of manipulating the timing of the spring moult by treatment with melatonin implants from December was investigated. As there is also evidence that the pineal plays a role in the seasonal regulation of body weight (Plotka *et al*, 1982) appetite (Forbes, 1986) and plasma prolactin concentrations (Pelletier, 1973) these were all monitored and their inter-relationships investigated. The thyroid hormones, thyroxine (T4) and triiodothyronine (T3) have been demonstrated to be involved in wool growth in sheep (Wallace, 1979a) and were also measured throughout the experiment. Both adult and juvenile goats were used to examine the response to melatonin treatment of both mature animals and those experiencing their first moult.

MATERIALS AND METHODS

Animals: The animals used were 20 juvenile (8 months of age) and 20 adult feral x dairy type (Toggenburg or Saanen) female goats. None of the animals had any history of hormone treatment or exposure to artificial photoperiod.

Management: From 11 December 1987 until 31 May 1988 the goats were individually penned on a slatted floor indoors under the influence of ambient photoperiod and temperature. They were offered *ad libitum* a complete pelleted diet (AA6, Wainman *et al,* 1975), which is based on barley and soya bean meal mixed with 30% barley straw. Fresh water was available at all times. They twice received an oral multi vitamin supplement (Pardevit, W.J. Dunlop,

Vet Suppliers) and an annual booster of Heptavac P (Hoechst, U.K.) as part of a routine Clostridial and Pastuerella vaccination programme. A broad spectrum anthelmintic (Oramec drench, Merck, Sharp and Dohme Ltd, U.K.) was administered at housing.

Treatments: The adult and juvenile goats were allocated in a balanced manner by live weight to two treatment groups, untreated controls and melatonin-treated animals. The treated animals received a series of subcutaneous implants of melatonin (Regulin Ltd, U.K., 18mg) on 11 December 1987, 1 February 1988, and 1 April 1988. Animals from each of the four groups (two treatments x two ages) were allocated to individual pens in blocks of four.

Measurements and sampling: The voluntary food intake (VFI) was estimated daily as the difference between the weight of food offered and that refused. Refusals were removed just prior to feeding and the weight to be offered was calculated from the previous day's intake. A refusal margin of 10% was allowed. Mean daily VFI's over each week for each animal were used in the statistical analyses. The animals were weighed fortnightly to the nearest 0.5kg. Blood samples (10ml) were collected weekly by jugular venepuncture into heparinized evacuated tubes. Samples were taken at the same time on each day, at 1100h. The samples were centrifuged at 2500 rpm for 20 minutes and the plasma removed and stored at -20 deg C.

Prolactin concentrations in plasma were determined using a double antibody radioimmunoassay (McNeilly and Andrews, 1974). The stock standard solution of prolactin (20 ug/ml), 125I label (NIAMDD-0PRL-15) and first antibody (Rabbit anti-ovine prolactin #50) were supplied by the MRC Unit of Reproductive Biology, Edinburgh. The standard solution was diluted in 1% bovine serum albumin phosphate buffer to give a range of standards from 0.4 to 200 ug/ml. The second antibodies, normal rabbit serum and donkey anti-rabbit serum

were provided by the Scottish Antibody Protection Unit, Glasgow. Intra and inter-assay coefficients of variation were less than 12.0 and 14.0% respectively. Plasma T3 and T4 concentrations were determined using radioimmunoassay kits from Amersham International Ltd. Intra assay coefficients of variation were less than 3.7% and 3.2% for T3 and T4 respectively and inter-assay coefficients less than 4.9% and 4.7% respectively. The standards provided ranged from 0- to 249 ng/ml for both T3 and T4.

To describe the cashmere content of the coat, patches of fibre ($10cm^2$) were measured on the mid-side position using callipers with arms $\sqrt{10}$cm apart and clipped using surgical clippers. The patches were clipped from different sites every four weeks from alternate sides of the animal. The samples were scored for content of cashmere fibres in reference to a photographic scale which ranged from 1 (no cashmere present) to 5 (cashmere more obvious than hair, sample very dense) (Plates II-VI). The samples were presented to the operator in a random manner to remove any preemptive bias.

Samples of skin were removed from the adult goats on the mid-side, every 4 weeks. Samples were removed under local anaesthetic (Lignocaine, W.J. Dunlop, Vet Suppliers) by the 'Snip' method after Ryder (1965). The skin samples after biopsy were immediately fixed in 10% formal saline. Serial sections of 8 microns were cut after the tissue had been processed and embedded in wax. Sections were stained using the 'sacpic' method (see Appendix for processing and staining schedules). Slides were viewed under a light microscope and sections at the level of the sebaceous gland were selected. Primary and secondary follicles were identified and classified as active or inactive and the proportion of follicles in each category calculated. Plates of each follicle type are described in the Appendix Plates VII-VIX.

Post treatment management: On 31 May 1988 the animals were turned out to

graze as one group at the Macaulay Land Use Research Institute's Sourhope Research Station, Roxburghshire. They were gathered weekly and blood sampled, weighed, fibre and skin sampled as above.

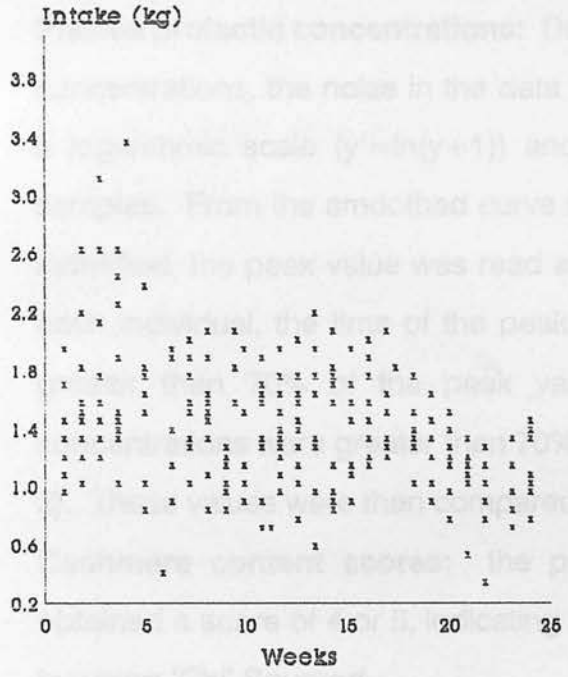
STATISTICAL ANALYSIS.

Voluntary Food Intakes: To smooth the effect of the observed daily fluctuations in VFI, daily values were averaged over each seven day period. On inspection of the data for individual animals (Figures 1a-d) it was clear that there was unequal variation in daily VFI values between the groups. As equal variance is an assumption of analysis of variance, the data were transformed on a logarithmic scale. Because some mean daily VFI's were less than 1, each value in the data set was increased by 1 prior to transformation ($y'=\ln(y+1)$). The transformed data were subjected to analysis of variance (Genstat 5.2.1 Lawes Agricultural Trust, Rothamsted Experimental station, 1990) firstly, considering time as a factor to demonstrate any differences between groups over the whole experimental period, and secondly, considering each week individually to determine whether significant differences were occurring between groups at different times during the experiment. As no pretreatment data were available, the linear change in VFI for each goat over the time course of the experiment, was described by the value of the regression coefficient of VFI on time (Genstat 5:2.1 Lawes Experimental Trust, Rothamstead Experimental Station, 1990).

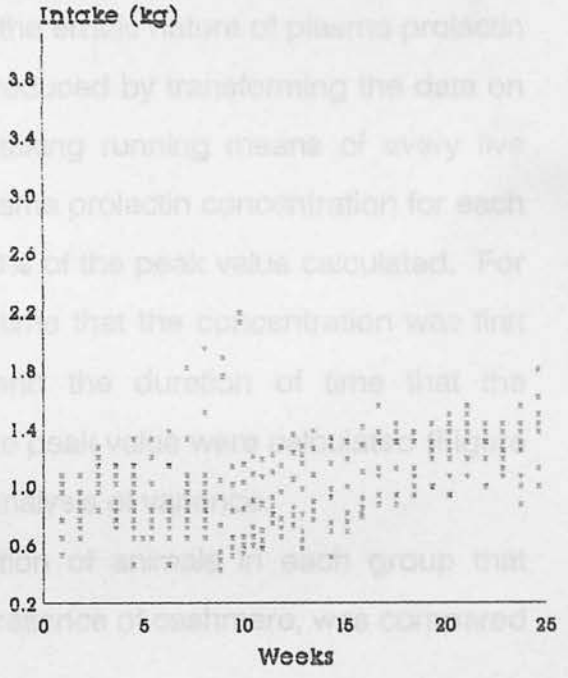
Live weight, plasma T3 and T4 concentration: These data demonstrated very little variance within groups and thus were not transformed as above. The data were tested for effects of treatment, age and time and their interactions by analysis of variance (Gentsat 5.2.1, Lawes Agricultural Trust, Rothamstead,

Figures 1a - d. Weekly mean/daily voluntary food intakes (kg) for each animal in each treatment in Experiment 1.

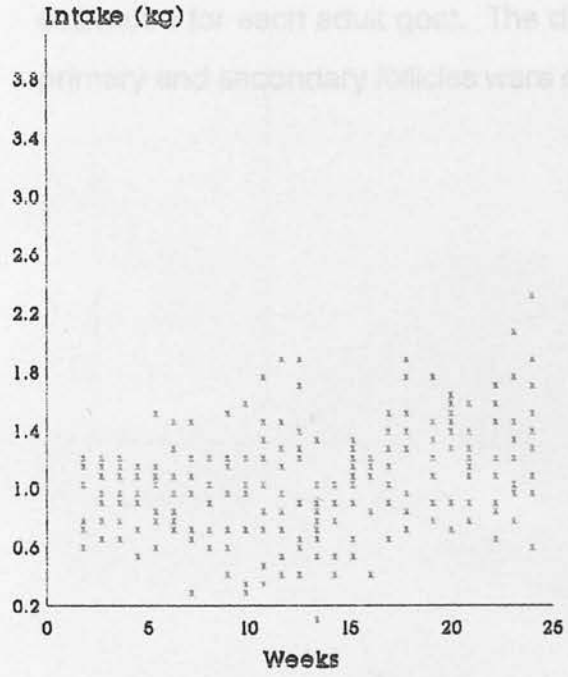
a) control adult treatment



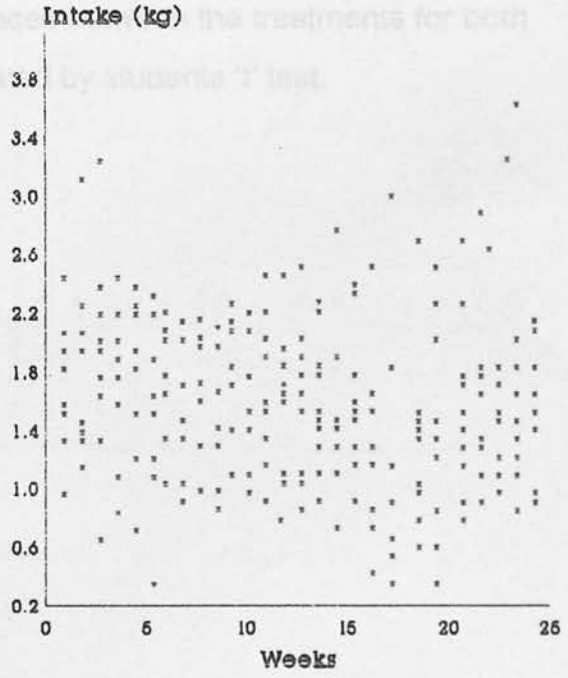
b) melatonin-treated adult treatment



c) control juvenile treatment



d) melatonin-treated juvenile treatment



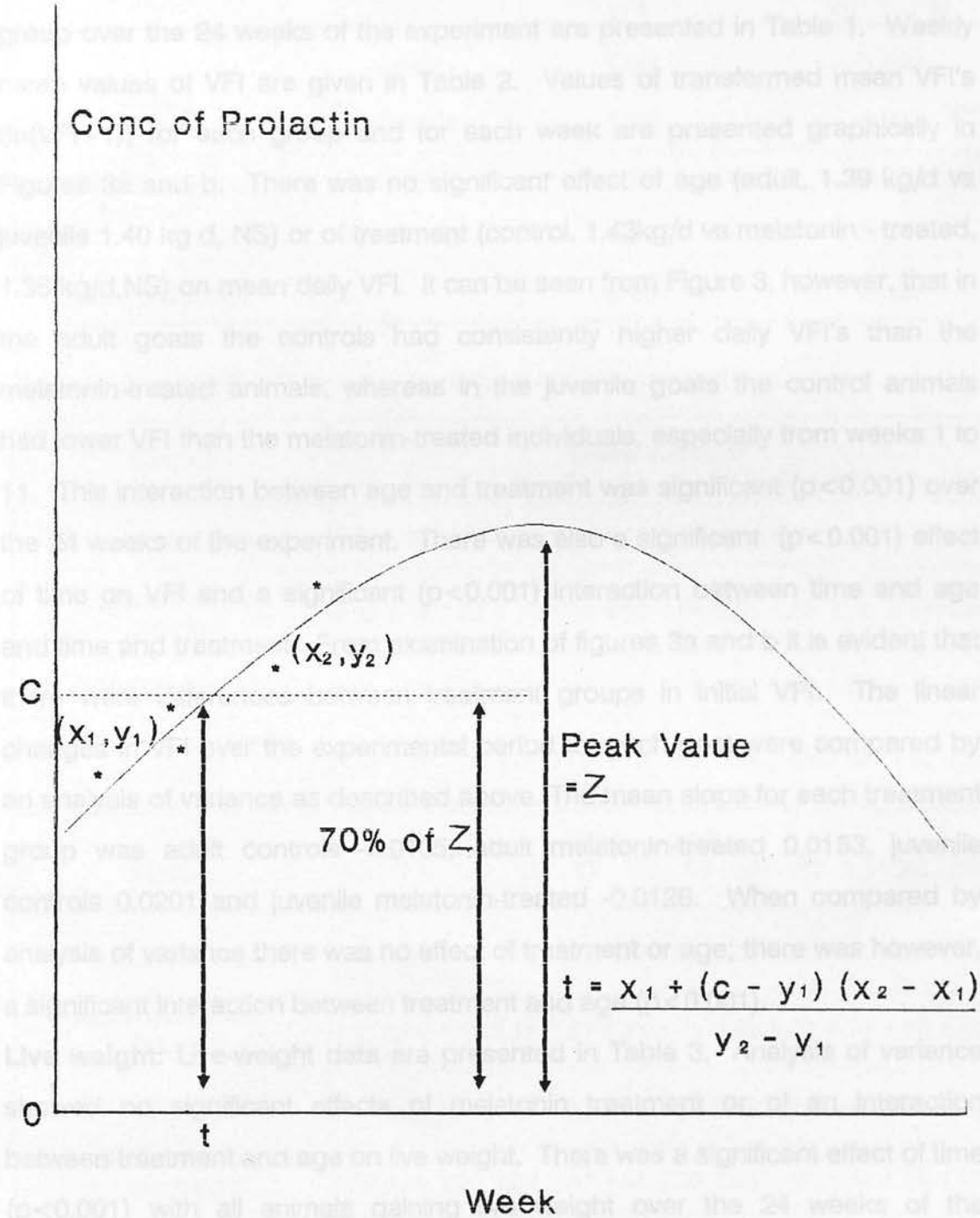
1990). The T3 and T4 data were tested for the complete experimental period and for each week individually.

Plasma prolactin concentrations: Due to the erratic nature of plasma prolactin concentrations, the noise in the data was reduced by transforming the data on a logarithmic scale ($y'=\ln(y+1)$) and by taking running means of every five samples. From the smoothed curve of plasma prolactin concentration for each individual, the peak value was read and 70% of the peak value calculated. For each individual, the time of the peak, the time that the concentration was first greater than 70% of the peak value, and the duration of time that the concentrations were greater than 70% of the peak value were calculated (Figure 2). These values were then compared by analysis of variance.

Cashmere content scores: the proportion of animals in each group that obtained a score of 4 or 5, indicating the presence of cashmere, was compared by using 'Chi' Squared.

Hair follicle status: The percentages of primary and secondary hair follicles in each sample that were observed to be in the active (anagen) state was estimated for each adult goat. The differences between the treatments for both primary and secondary follicles were estimated by students 't' test.

Figure 2. Calculation of the points on the x-axis which intercept 70% of the peak value



RESULTS

Voluntary food intakes: Mean daily VFI's (kg/day) for each treatment and age group over the 24 weeks of the experiment are presented in Table 1. Weekly mean values of VFI are given in Table 2. Values of transformed mean VFI's (ln(VFI+1)) for each group and for each week are presented graphically in Figures 3a and b. There was no significant effect of age (adult, 1.39 kg/d vs juvenile 1.40 kg d, NS) or of treatment (control, 1.43kg/d vs melatonin - treated, 1.36 kg/d,NS) on mean daily VFI. It can be seen from Figure 3, however, that in the adult goats the controls had consistently higher daily VFI's than the melatonin-treated animals, whereas in the juvenile goats the control animals had lower VFI than the melatonin-treated individuals, especially from weeks 1 to 11. This interaction between age and treatment was significant ($p<0.001$) over the 24 weeks of the experiment. There was also a significant ($p<0.001$) effect of time on VFI and a significant ($p<0.001$) interaction between time and age and time and treatment. From examination of figures 3a and b it is evident that there were differences between treatment groups in initial VFI. The linear changes in VFI over the experimental period for each goat were compared by an analysis of variance as described above. The mean slope for each treatment group was adult controls -0.0135, adult melatonin-treated 0.0153, juvenile controls 0.0201 and juvenile melatonin-treated -0.0126. When compared by analysis of variance there was no effect of treatment or age; there was however, a significant interaction between treatment and age ($p<0.001$).

Live weight: Live-weight data are presented in Table 3. Analysis of variance showed no significant effects of melatonin treatment or of an interaction between treatment and age on live weight. There was a significant effect of time ($p<0.001$) with all animals gaining live weight over the 24 weeks of the

Table 1. Mean daily VFI of each treatment and age group averaged over the complete experimental period.

Treatment	Age	Mean VFI and s.e. from weeks 1-24 (kg/day) VFI	ln(VFI+1)
Control	Adult	1.59	0.941
	Juvenile	1.26	0.811
Melatonin treated	Adult	1.20	0.781
	Juvenile	1.54	0.917
Standard Error		0.66	0.025

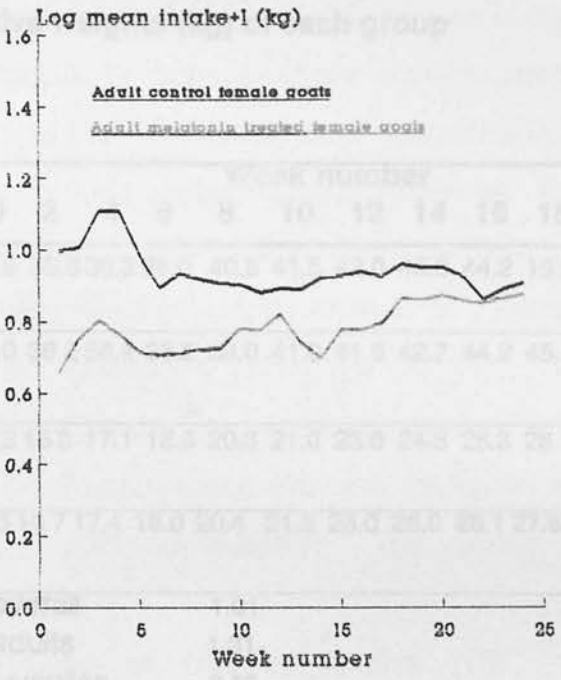
Table 2. Mean daily VFI (kg/day) of each group from weeks 1 - 24.

Group	Week number 1	2	3	4	5	6	7	8	9	10	11	12
Adult control	1.66	1.74	2.04	2.04	1.68	1.45	1.53	1.50	1.48	1.46	1.41	1.44
Adult melatonin treated	0.93	1.12	1.23	1.15	1.04	1.02	1.04	1.05	1.06	1.18	1.17	1.27
Juvenile control	1.06	1.09	1.16	1.15	1.13	1.12	1.05	1.11	1.17	1.23	1.22	1.30
Juvenile melatonin treated	1.75	1.73	1.79	1.75	1.66	1.59	1.42	1.58	1.53	1.54	1.50	1.53

Group	Week number 13	14	15	16	17	18	19	20	21	22	23	24
Adult control	1.43	1.51	1.52	1.55	1.52	1.61	1.59	1.59	1.52	1.36	1.43	1.48
Adult melatonin treated	1.10	1.00	1.17	1.17	1.21	1.37	1.36	1.39	1.35	1.34	1.37	1.40
Juvenile control	1.13	1.12	1.23	1.28	1.31	1.42	1.49	1.47	1.43	1.46	1.52	1.51
Juvenile melatonin treated	1.30	1.06	1.28	1.35	1.33	1.45	1.38	1.46	1.49	1.54	1.56	1.63
mean s.e.d.	overall 0.066 adults 0.070 juveniles 0.115											

Figures 3a and b. Mean VFI's over the coarse of the experiment for both adult and juvenile goats expressed as log (mean VFI + 1) values.

a) adult cashmere goats



b) juvenile cashmere goats

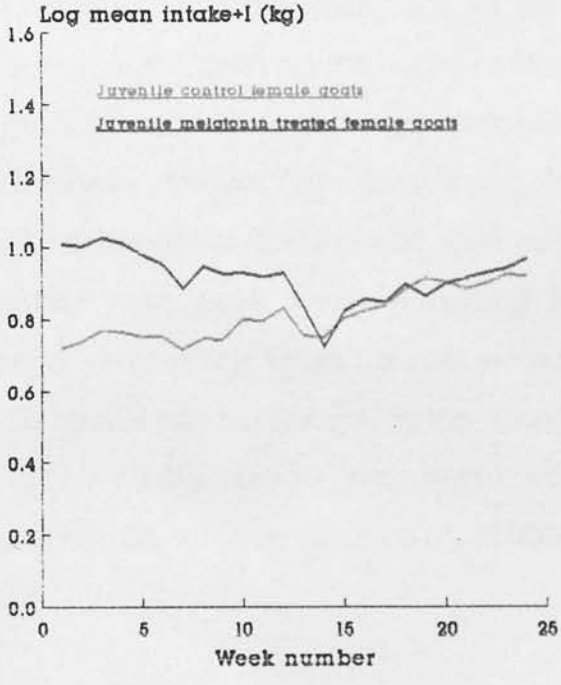


Table 3. Mean live weights (kg) of each group

Group	Week number 0	2	4	6	8	10	12	14	16	18	20	22	24	26
Adult Controls	36.6	36.6	36.3	38.0	40.5	41.5	42.0	43.3	44.2	15.3	46.5	47.0	49.2	45.5
Adult Melatonin	36.0	36.2	36.4	38.5	39.0	41.0	41.5	42.7	44.2	45.5	46.0	47.1	48.4	46.0
Juvenile Controls	16.2	16.8	17.1	18.5	20.3	21.0	23.0	24.8	26.3	28.1	29.0	29.9	31.9	29.1
Juvenile Melatonin	16.3	16.7	17.4	18.0	20.4	21.5	23.0	25.0	26.1	27.6	28.5	29.6	31.2	28.4
mean s.e.d.	overall				1.01									
	adults				1.31									
	juveniles				0.55									

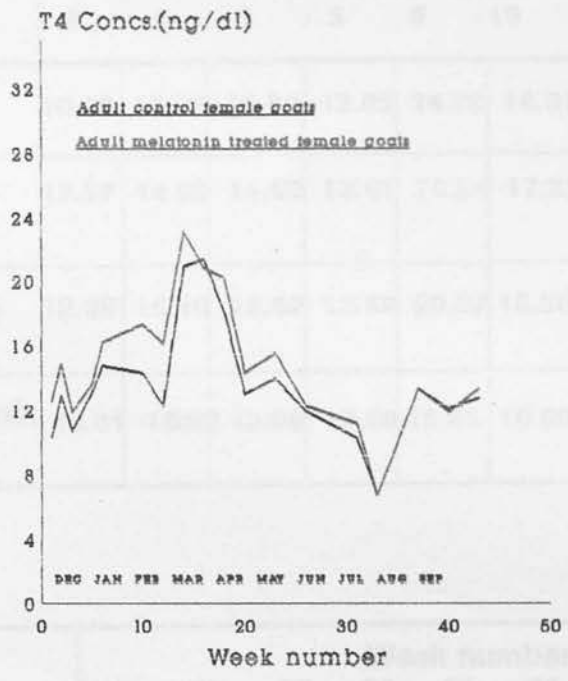
experiment.

Plasma concentrations of thyroxine: (T4). Figure 4 shows the mean plasma concentrations of T4 for each age group. There was a significant effect of time ($p<0.001$) with plasma T4 concentrations demonstrating a similar cyclical pattern for all four groups. In general, peak concentrations occurred between weeks 14 and 16 with lowest concentrations in week 33. There was a significant interaction between age of animal and time ($p<0.001$), reflecting the more extreme changes exhibited by juvenile animals in Plasma T4 concentrations, with higher peak concentrations and lower minimum concentrations of T4. When the data were examined on a weekly basis, the juvenile animals had significantly lower T4 concentrations than the adults on weeks 18, 33 and 43 ($P<0.001$, 0.01 and 0.001 respectively). The means for each group are presented in Table 4.

Plasma concentrations of triiodothyronine (T3). Mean plasma T3 concentrations for each group are presented in Table 5 and Figure 5. There was no significant effect of age or treatment on plasma concentration of T3 when the data over the complete experimental period are considered. Plasma concentrations of T3 did not demonstrate the cyclical pattern that was observed in the concentrations of T4 but there was a significant effect of time ($p<0.001$) and an interaction between time and age ($p<0.001$). In the adult goats two peaks were observed in December-January and June-July. The juvenile goats had a different pattern with peak levels occurring in January and the concentration thereafter decreasing. When the data were analysed on a weekly basis by age the adult goats had significantly higher plasma T3 concentrations on weeks 1 and 31 ($p<0.001$, 0.001) and significantly lower plasma T3 concentrations on weeks 12, 16 and 18 ($p<0.01$, 0.001, 0.001, respectively) than juvenile goats.

Figures 4a and b. Mean plasma concentrations of Thyroxine (T4) for each group.

a) adult cashmere goats



b) juvenile cashmere goats

Table 4. Mean plasma Thyroxine (T_4) concentration (ng/dl)for each group.

Group	Week number 1	2	3	5	6	10	12	14	16
Adult control	10.29	12.97	10.69	13.05	14.78	14.31	1.31	20.93	21.41
Adult melatonin treated	12.57	14.92	11.92	13.61	16.24	17.32	16.15	23.09	20.90
Juvenile control	12.99	15.10	12.52	13.59	20.07	18.56	16.29	22.72	23.59
Juvenile melatonin treated	10.31	13.82	12.08	13.60	19.45	16.60	18.17	22.48	22.60

Group	Week number 18	20	23	26	31	33	37	40	43
Adult control	18.14	13.02	14.02	11.98	10.22	6.69	13.32	12.10	12.77
Adult melatonin treated	20.22	14.35	15.58	12.29	11.16	6.72	13.31	11.90	1.35
Juvenile control	15.34	15.47	15.75	10.85	10.58	5.16	13.85	12.11	9.57
Juvenile melatonin treated	15.14	14.28	14.64	11.02	12.58	5.97	13.42	12.56	6.95
mean s.e.d. overall			0.551						
adults			0.593						
juveniles			0.595						

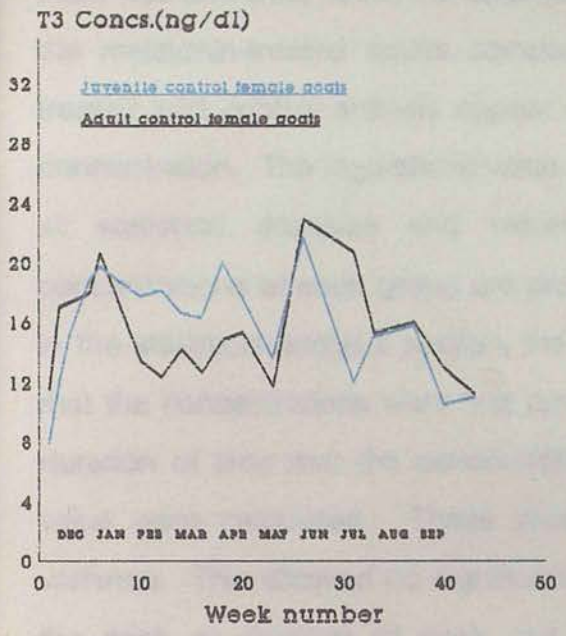
Table 5. Mean plasma Triiodothyronine (T3) concentration (ng/dl)for each group.

Group	Week number 1	2	3	5	6	10	12	14	16
Adult control	11.44	17.01	13.94	17.92	20.63	13.42	12.26	14.21	12.62
Adult melatonin treated	11.66	16.38	13.81	16.75	18.93	11.95	14.41	16.08	12.87
Juvenile control	7.88	11.89	15.06	18.82	19.67	17.69	18.14	16.60	16.33
Juvenile melatonin treated	9.54	14.3	17.54	22.34	22.38	15.89	16.81	15.93	15.68

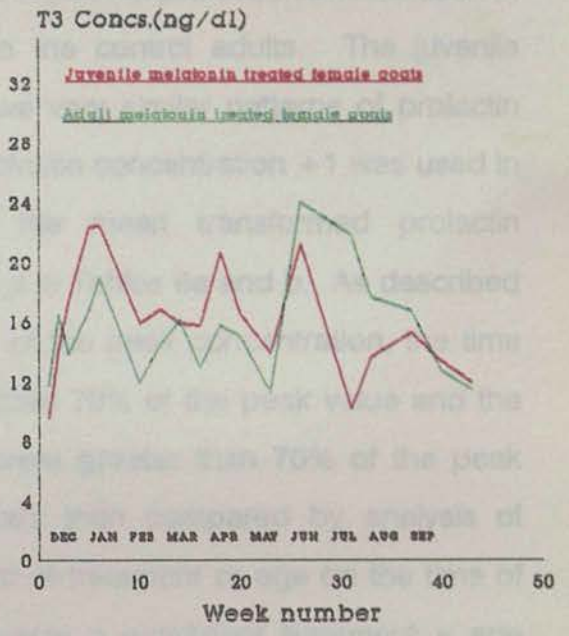
Group	Week number 18	20	23	26	31	33	37	40	43
Adult control	14.85	15.28	11.69	22.88	20.68	15.15	16.11	12.85	11.19
Adult melatonin treated	15.67	14.97	11.33	24.0	21.76	17.63	16.70	12.68	11.42
Juvenile control	19.97	17.64	13.63	21.66	12.03	15.04	15.84	10.54	11.02
Juvenile melatonin treated	20.60	16.51	13.94	21.18	10.13	13.65	15.22	13.26	11.89
mean s.e.d. overall			0.446						
adults			0.732						
juveniles			0.605						

Figures 5a - d. Mean plasma concentrations of Triiodothyroninine (T3) for each group.

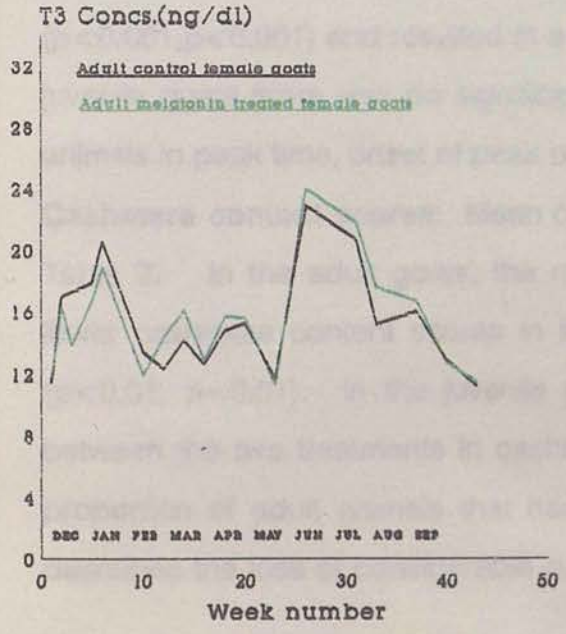
a) adult and juvenile control goats



b) adult and juvenile melatonin treated goats



c) adult control/melatonin treated goats



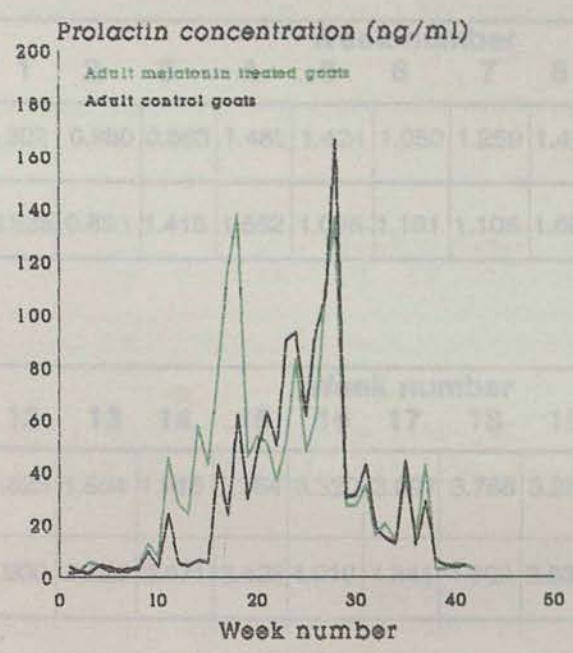
d) juvenile control/melatonin treated goats

Plasma concentrations of prolactin: Graphs of mean prolactin concentration were drawn for each group of animals (Figures 6a and b). These indicated that there was an earlier rise in the seasonal peak of plasma prolactin concentration in the melatonin-treated adults compared to the control adults. The juvenile treated and control animals appear to have very similar patterns of prolactin concentration. The logarithmic value of prolactin concentration +1 was used in all statistical analyses and values of the mean transformed prolactin concentrations of each group are presented in Tables 6a and b. As described in the statistical analysis section, the time of the peak concentration, the time that the concentrations were first greater than 70% of the peak value and the duration of time that the concentrations were greater than 70% of the peak value were calculated. These values were then compared by analysis of variance. This showed no significant effect of treatment or age on the time of the peak or duration of peak, but did show a significant treatment x age interaction in the onset of peak ($p<0.001$). To investigate these data further, the two age groups were analysed separately. In the adult goats, melatonin treatment significantly advanced the peak time and the onset of the peak ($p<0.001$,$p<0.001$) and resulted in a longer duration of peak ($p<0.001$). In the juvenile goats there was no significant difference between control and treated animals in peak time, onset of peak or duration of peak.

Cashmere content scores: Mean cashmere content scores are presented in Table 7. In the adult goats, the melatonin-treated animals had significantly lower cashmere content scores in March and April than the control animals ($p<0.01$, $p<0.01$). In the juvenile goats there was no significant difference between the two treatments in cashmere content score. The decrease in the proportion of adult animals that have a score 4 or 5 for cashmere content, describes the loss of considerable quantities of cashmere from the coat and is

Figures 6a and b. Mean plasma concentrations of prolactin.

a) adult control/melatonin treated goats



b) juvenile control/melatonin treated goats

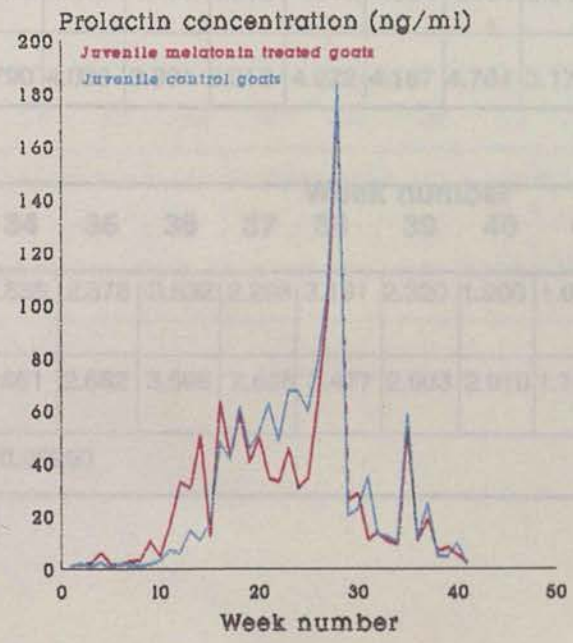


Table 6a. Mean weekly plasma prolactin concentration of adult goats, expressed as log values +1 (ng/ml).

Group	Week number 1	2	3	4	5	6	7	8	9	10	11
Adult control	1.302	0.980	0.863	1.485	1.424	1.050	1.259	1.420	1.935	1.587	2.619
Adult melatonin	0.925	0.893	1.415	1.552	1.086	1.191	1.105	1.607	2.184	1.774	3.396

Group	Week number 12	13	14	15	16	17	18	19	20	21	22
Adult control	1.823	1.504	1.916	1.764	3.320	3.097	3.766	3.289	3.727	4.086	3.827
Adult melatonin	2.900	2.926	3.671	3.428	4.010	4.341	4.609	3.631	3.726	3.711	3.405

Group	Week number 23	24	25	26	27	28	29	30	31	32	33
Adult control	4.359	4.374	4.033	4.303	4.300	4.534	4.064	3.317	3.309	3.126	2.769
Adult melatonin	3.790	4.030	3.804	3.915	4.072	4.187	4.784	3.174	3.192	3.045	2.772

Group	Week number 34	35	36	37	38	39	40	41	42	43	44
Adult control	2.535	2.378	3.532	2.293	3.191	2.320	1.900	1.614	1.104	0.947	0.966
Adult melatonin	2.851	2.682	3.595	2.685	3.477	2.603	2.010	1.792	1.293	1.436	1.286
mean s.e.d.	0.09260										

Table 6b. Mean weekly plasma prolactin concentration of juvenile goats expressed as log values + 1 (ng/ml).

Group	Week number 1	2	3	4	5	6	7	8	9	10	11
Juvenile control	0.705	0.976	0.643	0.963	0.469	1.014	0.780	0.791	0.991	1.381	1.946
Juvenile melatonin	0.846	0.932	1.011	1.767	0.898	0.899	1.058	1.331	1.947	1.506	2.436

Group	Week number 12	13	14	15	16	17	18	19	20	21	22
Juvenile control	1.888	1.662	1.996	2.218	3.094	3.149	3.416	3.507	3.836	4.160	3.715
Juvenile melatonin	2.876	2.558	3.227	2.342	3.783	3.480	3.682	3.401	3.500	3.238	3.269

Group	Week number 23	24	25	26	27	28	29	30	31	32	33
Juvenile control	3.913	3.971	3.908	4.263	4.350	4.487	5.202	2.895	3.035	3.023	3.480
Juvenile melatonin	3.511	3.303	3.483	4.129	4.260	4.328	5.167	3.134	3.249	2.344	2.535

Group	Week number 34	35	36	37	38	39	40	41	42	43	44
Juvenile control	2.410	2.368	3.823	2.402	2.867	1.711	1.662	2.155	1.055	0.975	0.936
Juvenile melatonin	2.183	2.311	3.751	2.366	2.905	1.931	2.123	1.735	1.125	1.107	1.793
mean s.e.d.	0.1074										

Table 7. Mean cashmere content score (s.e.) for each treatment group during the experiment.

Group	Date					
	15 dec	12 jan	9 feb	8 mar	5 apr	3 may
Adult controls	4.9 (0.32)	4.5 (0.53)	4.6 (0.52)	4.2 (0.79)	3.1 (0.99)	1.6 (1.07)
Adult melatonin	4.5 (0.53)	4.7 (0.48)	4.3 (0.48)	3.5 (0.53)	2.1 (0.74)	1.3 (0.67)
Juvenile controls	4.7 (0.48)	4.8 (0.42)	4.3 (0.67)	4.0 (0.94)	2.9 (1.29)	1.3 (0.48)
Juvenile melatonin	5.0 (-)	5.0 (-)	4.6 (0.52)	3.7 (0.90)	2.7 (1.20)	1.9 (1.1)

indicative that they have been moulting. From Figure 7 it is evident that there was an earlier decrease in the number of animals scoring 4 or 5 in March and April in the melatonin-treated adults compared to the controls.

Hair follicle activity: The mean proportion of primary and secondary follicles that were classified as active, in the adult control and treated groups are presented in Tables 8a and b. There was a significantly higher proportion of primary follicles in the active state in the melatonin treated adults compared to the controls on 3 May 1988 ($p<0.001$) and 31 May 1988 ($p<0.005$). For the secondary follicles there was also a significantly higher proportion of follicles in the active state in the melatonin treated animals on 3 May 1988 ($p<0.005$) and 31 May 1988 ($p<0.001$) compared to the control animals.

Figure 7. Change in proportion of animals in each group that scored 4 or 5.

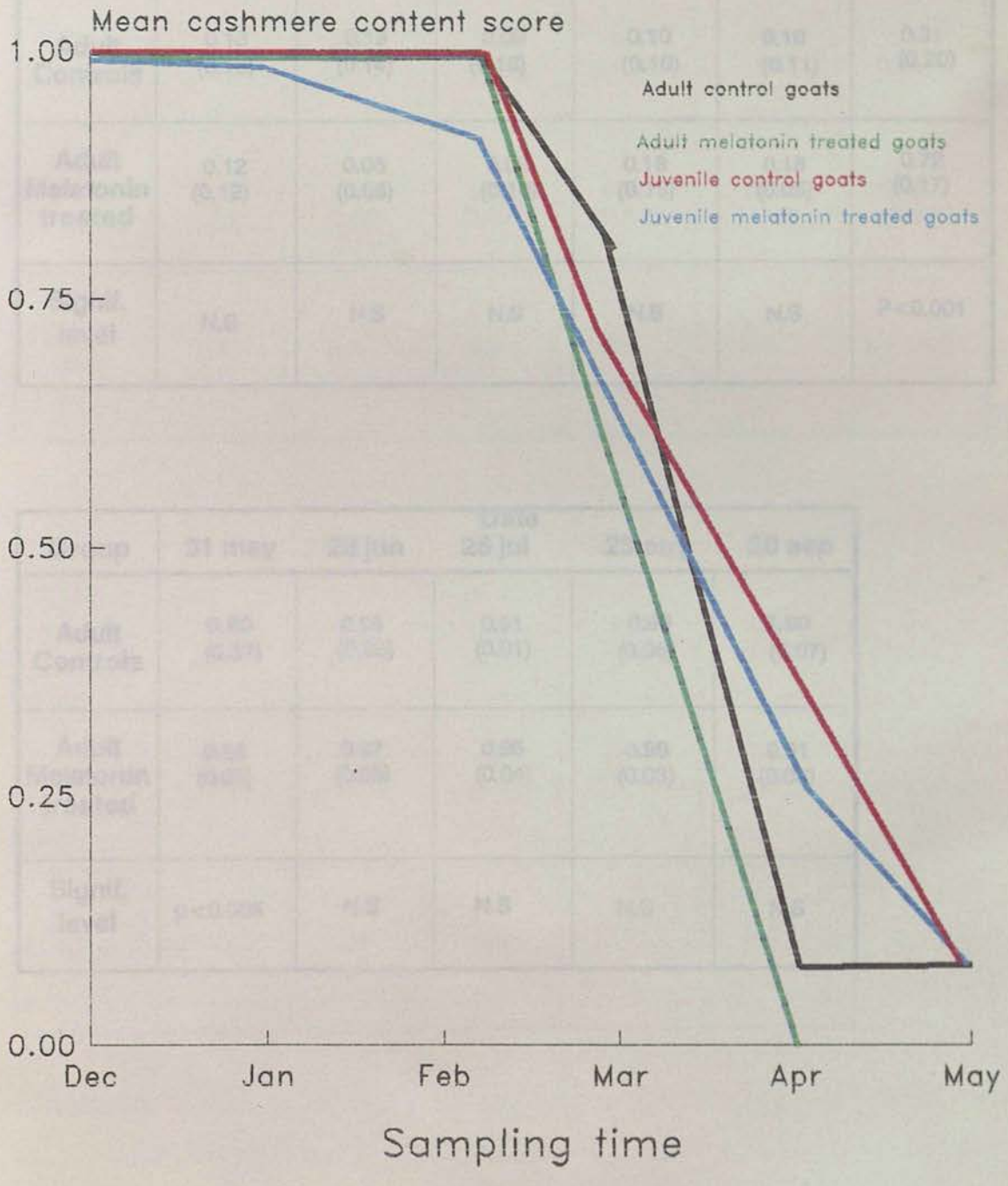


Table 8a. Mean proportion of primary follicles in the active state in adult goats (s.e. in parenthesis).

Group	Date 15 dec	12 jan	9 feb	8 mar	5 apr	3 may
Adult Controls	0.13 (0.15)	0.18 (0.14)	0.09 (0.16)	0.10 (0.10)	0.16 (0.11)	0.31 (0.20)
Adult Melatonin treated	0.12 (0.12)	0.06 (0.05)	0.08 (0.10)	0.18 (0.19)	0.18 (0.05)	0.72 (0.17)
Signif. level	N.S	N.S	N.S	N.S	N.S	P<0.001

Group	Date 31 may	28 jun	26 jul	23 aug	20 sep
Adult Controls	0.60 (0.37)	0.95 (0.05)	0.91 (0.01)	0.96 (0.06)	0.90 (0.07)
Adult Melatonin treated	0.98 (0.05)	0.97 (0.03)	0.96 (0.04)	0.99 (0.03)	0.91 (0.08)
Signif. level	p<0.005	N.S	N.S	N.S	N.S

Table 8b. Mean percentage of secondary follicles in the active state in the adult goats (s.e. in parenthesis)

Group	Date 15 dec	12 jan	9 feb	8 mar	5 apr	3 may
Adult Controls	0.27 (0.32)	0.03 (0.03)	0.07 (0.08)	0.14 (0.13)	0.26 (0.12)	0.31 (0.23)
Adult Melatonin treated	0.29 (0.31)	0.02 (0.02)	0.04 (0.05)	0.28 (0.33)	0.30 (0.12)	0.63 (0.20)
Signif. level	N.S	N.S	N.S	N.S	N.S	$p<0.005$

Group	Date 31 may	28 jun	26 jul	23 aug	20 sep
Adult Controls	0.35 (0.30)	0.80 (0.13)	0.88 (0.15)	0.91 (0.18)	0.89 (0.21)
Adult Melatonin treated	0.89 (0.12	0.92 (0.11)	0.87 (0.16)	0.93 (0.05)	0.91 (0.08)
Signif. level	$p<0.001$	N.S	N.S	N.S	N.S

DISCUSSION

Voluntary food intakes

A winter depression in appetite occurs in several ruminant species such as sheep and deer (Kay, 1979, 1985). The more primitive breeds of sheep, such as the Soay and the Scottish Blackface, show a greater amplitude in their appetite cycle than the more domesticated breeds for example lowland English breeds (Forbes, 1982; Kay, 1985; Iason and Mantecon, 1991). There is no published literature concerning the presence or otherwise of seasonal cycles of appetite in the goat. However, as goats demonstrate strongly seasonal cycles of both reproduction and hair growth it is probable that they may also exhibit an annual cycle of appetite.

Diurnal and circannual cycles of appetite in sheep have been partially associated with changes in melatonin secretion. Schanbacher and Crouse (1981) reported that sheep eat less frequently and in smaller amounts during the hours of darkness, when melatonin synthesis and secretion is at it's highest. Kay (1979) demonstrated that compressing the annual cycle of daylength into 6 months resulted in a concomitant acceleration of the food intake cycle. In a review of changes in appetite between animals on long days (16L:8D) compared to short days (8L:16D) Forbes (1982) concluded that both growth and intake were stimulated under a long-day environment and that the effect on intake was a response to an increase in growth rate.

As the aim of this experiment was to alter the timing of the spring moult by manipulating plasma melatonin levels it was important to measure any responses in VFI and live weight to ensure that no detrimental effects on juvenile growth rate or adult maintenance of body weight occurred in response to the treatment. Spillage of feed occurred in all animals, and as they were

housed on slats this could not be measured. It can be assumed, however, that this behaviour was exhibited by individuals in all groups rather than only those in one group.

An initial rise in VFI was observed in all four groups, which was possibly due to the animals becoming accustomed to a new environment and the complete pelleted diet. The slopes of the change in VFI over time demonstrate an increase in VFI in the melatonin treated adults and the juvenile controls and a decrease in VFI in the adult controls and the melatonin-treated juveniles. However, if we visually assess the slope of Figures 2c and 2b, it becomes apparent that the melatonin-treated juveniles demonstrated a decrease in VFI over the first 14 weeks and thereafter VFI began to rise, as did the adult controls from week 6. This does not suggest an obvious seasonal increase in appetite as was demonstrated in sheep by Forbes (1982), between September and January or by Iason and Mantecon (1991) over the winter months. A rise in VFI has also been observed in deer between January and May (Loudon *et al,* 1989). The melatonin-treated adult goats also demonstrated an initial rise in VFI followed by a small increase over the experimental period. This indicates that the melatonin treatment in the goat does not necessarily suppress appetite as has been found in deer (Milne *et al,* 1990).

It cannot be concluded from these results that goats do or do not have a seasonal cycle of appetite regulated by changes in melatonin synthesis and secretion as the timing of the treatment may alter the response to an increased duration of melatonin. During long days the differences between control and melatonin treated animals in the duration of melatonin secretion are greater and it is possible that more obvious differences may be observed at this time.

The juvenile goats were 8 months of age at the beginning of the experiment. The control juvenile goats demonstrated an increase in VFI over the

experimental period. The melatonin-treated juveniles showed a decrease in appetite in the first half of the experiment followed by a steady increase at the same rate as the controls in the second half of the experiment. The live weights of both groups of juveniles increased at the same rate over the experiment. As there are no differences between the melatonin-treated and control adults in VFI or live weight it can be concluded that any differences observed in hormone profiles or hair follicle changes in the adults are not a result of differences in nutritional status. This is also true for the juvenile goats as no significant differences were observed in VFI or live weight. A significant interaction between age and treatment was however observed. This difference in response to melatonin treatment may be explained by the dose of melatonin administered per kg of live weight and/or the different reproductive status between the adults and the juveniles. Steroids have long been associated with appetite control and may be involved in the response to seasonal regulation (Muir *et al,* 1972). These results pose some interesting questions about the interactions between puberty, and the seasonal control of appetite modulated by melatonin, it is however beyond the scope of this experiment to draw any firm conclusions about those interactions.

Thyroid hormones

Evidence for the role of the thyroid hormones, T3 (triiodothyronine) and T4 (thyroxine) in the control of wool growth in sheep has existed for some time (Wallace, 1979a). Ferguson *et al* (1965) and Girard (1979b) both report that hypophysectomy, which prevents TSH (Thyroid stimulating hormone) release, suppresses wool growth in sheep and after a period of weeks, wool follicles eventually reach a stage indistinguishable from telogen.

Thyroidectomy, in sheep maintained on a constant diet, also reduces wool

growth, although in this case only to 60% of preoperative rates (Theriez and Rougeot, 1962, Ferguson *et al,* 1965). The reduction in fibre growth rate is entirely due to a reduction in fibre length growth rate, fibre diameter is unchanged (Theriez and Rougeot, 1962). As observed in the hypophysectomised sheep, administration of T4, restores wool growth rate to pre-operative rates. In contrast to hypophysectomised sheep however, if the dose of T4 is increased, wool growth rates above pre-operative rates can be obtained in thyroidectomised (Ferguson *et al*, 1965) and normal sheep (Labban, 1957, Ferguson, 1958). this suggests that some pituitary factor, other than TSH is necessary for thyroid hormones to express fully their physiological potential (Wallace, 1979).

Although thyroid hormones have been shown to increase wool growth rate, plasma concentrations of thyroid hormones have not been found to be associated with natural changes in wool growth rate (Wallace, 1976). Reklewska (1975), for example, demonstrated that changes in wool growth associated with changes in ambient temperature or shearing were not accompanied by changes in plasma T4 concentrations.

Both thyroid hormones, T4 (thyroxine) and T3 (triiodothyromine), were determined in this experiment. It is generally accepted that T3 is the biologically active thyroid hormone and that T4 is converted to T3 (Fisher *et al,* 1972; Abrams and Larsen, 1973; Vriend *et al,* 1983). The cyclic pattern which was observed for T4 concentration in goats in all treatment groups is similar to that described for sheep by Wallace (1979a), who reported peak plasma T4 concentrations in spring and the lowest concentrations in autumn. Neither melatonin treatment nor age had any effect on plasma T4 concentrations. There was also no consistent difference between controls and melatonin-treated adults or juveniles in plasma T3 concentrations. In the adult goats,

however, two peaks in plasma T3 concentration, one in late winter and one in summer, were observed. Whereas in the juveniles there was one peak in late winter followed by a reduction in plasma T3 concentrations thereafter. These differences may be due to differences in the growth rate of juvenilescompared to adults. No differences were observed between control and melatonin-treated animals and it can be concluded that melatonin treatment from December until May, does not affect T4 or T3 concentrations in the goat. Also the peaks did not correspond to periods of change in the hair follicle cycle and it can be concluded that the thyroid hormones are not involved in regulating the seasonal spring moult.

Plasma prolactin concentrations.

A cyclic pattern of plasma prolactin concentrations with peak levels in summer and basal levels in winter was demonstrated in all treatment groups. This closely corresponds to the annual cycle reported by Buttle (1974) in male goats.

Plasma prolactin concentration has been suppressed by melatonin administration in sheep (Kennaway *et al,* 1982a and b, Lincoln and Ebling, 1985), mink (Rose *et al* , 1985) and blue foxes (Smith *et al*, 1987a). When the treatment was imposed during the summer in blue foxes for one year, testicular regression and the moult to a summer coat was prevented. In mink, melatonin implanted in January or March led to a 3-month delay in the normal decrease in body weight and testes volume, the increase in plasma prolactin concentrations and the onset of the spring moult (Allain *et al,* 1981).

In this experiment, melatonin treatment in the adult did not suppress plasma prolactin concentration and indeed significantly advanced the time of the peak in concentration. Implantation of melatonin in December is similar to the

imposition of extended periods of short days or constant darkness. When animals are held in short daylength or constant darkness for long periods of time they exhibit spontaneous changes in plasma prolactin concentrations, such that they are no longer correlated with the prevailing photoperiod. This phenomenon is termed photorefractoriness. As well as animals showing refractoriness to photoperiod, they can also show refractoriness to the effects of exogenous melatonin (Bittman, 1978). Almeida and Lincoln (1984) also reported that during refractoriness to short day photoperiod plasma melatonin levels were disrupted and did not reflect the pattern of light and dark. The combination of a period of short days and administration of a constant release melatonin implant in the adult goats also resulted in refractoriness to the melatonin signal. The timing of the melatonin treatment must be crucial in this regard since Allain *et al* (1989) did not observe such an effect in mink when the only difference in melatonin treatment was a later start by a month in imposing the treatment. The plasma prolactin concentration of the juveniles did not respond in the same way as the adults to melatonin treatment. The juveniles would be experiencing their first short days, excluding prenatal information. It is possible that the plasma prolactin concentrations in the first months are predetermined during foetal exposure via the mother and that they are less easy to manipulate than the adults. It has been demonstrated in the hamster, and sheep that information regarding the prevailing photoperiod experienced by the mother during gestation is transferred to the young (hamsters, Stetson *et al*, 1986, sheep, Seron-Ferre *et al*, 1989). It must also be noted that the differences in dose of melatonin per kg of liveweight, VFI and reproductive status may all be involved in the response to melatonin treatment.

Cashmere fibre loss

The cashmere content score is an estimate of the quantity of cashmere present in the animal's coat at the time of sampling. The change in score from 5 (winter type coat) to 1 (sparse summer coat) during the experiment relects the time and rate of moult to summer pelage. All treatment groups demonstrated a gradual decline in cashmere content score during the experiment. Within each group, moulting took place over several months, for example, from February until May in the control group and over several weeks in individual animals. The asynchrony in the response of the hair follicles in an individual animal, once the moult has commenced, indicates that there are differences in the ability of individual follicles to respond to an endocrine signal. There are obvious advantages to the animal in having a degree of asynchrony over the body to allow a gradual change in the thermal qualities of the coat, rather than a sudden change. From the cashmere content scores it is evident that there was an earlier decline in the score in the melatonin treated adults compared to the other treatment groups and this was associated withan earlier advance in plasma prolactin concentration.

Histological status.

The proportion of hair follicles in an active state at any one time is indicative of the timing of the the growth of the replacement coat and is associated with the timing of the moult. For both the primary and secondary follicles there was a higher proportion of active follicles on 3 May ($p<0.001$, primary; $p<0.01$, secondary) and 31 May ($p<0.01$, primary; $p<0.001$, secondary) in the adult melatonin-treated group compared to the adult controls. This supports the evidence from cashmere content scores that moulting was advanced in the melatonin-treated adults, and resulted in an earlier establishment of the summer

coat. As the melatonin-treated adults also had an advance in the spring rise in plasma prolactin concentration it is proposed that there is an association between the timing of the rise in plasma prolactin concentration and the timing of the spring moult. The advance in both expulsion of the mature fibre from the follicle (from the proportion of active follicles) was observed in both the primary and secondary follicles. This indicates that both the primary and secondary follicles are responding to the same endocrine signal. It is proposed that the rise in plasma prolactin concentration observed in the spring, is involved in a chain of events that ultimately control moulting and the initiation of new growth. This hypothesis is supported by the work of Allain *et al* (1981) in mink and by Duncan and Goldman (1984) in the Djungarian hamster. To establish clearly if prolactin does have a role in initiating hair follicle activity it is necessary to both delay and advance the rise in prolactin concentration and study the effect on hair folicle activity.

As the response to melatonin treatment in the adults, i.e. the advance in the spring rise in plasma prolactin concentration, was contradictory to the response to melatonin treatment previously observed in mink (Allain *et al*, 1981) and Blue-foxes (Smith *et al*, 1987a), it is necessary to ensure that the response was due to the timing of the administration of the melatonin or the species of animal used, rather than inefficient release of melatonin from the implant. This would be demonstrated clearly by the administration of the implants (Regulin, 18mg) to adult female goats, at a time when endogenous levels of prolactin are high. The response of plasma prolactin concentration to melatonin tratment during long days has been well documented (Lincoln and Ebling, 1985). Implants of melatonin given during long days, switch the animal to a short day response, i.e. in the Soay ram a decrease in plasma prolactin concentration and an increase in testes size is observed.

It was concluded from this study, that there may be an association between the time of the rise in plasma prolactin concentration and the onset of the moult, however the use of melatonin implants in the winter and spring did not successfully manipulate prolactin levels. In the next experiment, another method of controlling prolactin synthesis and release is investigated, and the melatonin implants are tested during long days.

Chapter Three: Response of plasma prolactin concentration to one of three dose levels of bromocriptine (Parlodel L.A.) or 18mg of melatonin (Regulin) in the spring.

Experiment Two: Response of plasma prolactin concentration to one of three dose levels of bromocriptine (Parlodel L.A.) or 18mg of melatonin (Regulin) in the spring.

INTRODUCTION

A long-acting bromocriptine preparation (Parlodel L.A., Sandoz Pharmaceuticals, Basle, Switzerland) has been successfully used in the prevention and suppression of puerperal lactation in women (Rolland *et al*, 1986) via the suppression of plasma prolactin concentrations. Clinical trials in post-partum women indicated that 50mg of Parlodel L.A. administered as a single injection (i.m.) inhibited or suppressed lactation and was well tolerated systemically and locally (Rolland *et al*, 1986).

Forsyth *et al* (1985) i.m. injected British Saanen goats from week 8 until week 20 of pregnancy with 5mg of bromocriptine per day. Plasma prolactin concentrations were suppressed to less than 0.4ug/l in 60% of samples and to less than 2ug/l in 96% of samples. These values were significantly lower than that of the control animals ($p<0.001$).

In other species dose levels of 0.1mg/day in dogs (Cancannon *et al*, 1987) 1mg/day (s.c.) and 0.5mg/12 hours (i.m.) in mink (Martinet *et al*, 1984) have been found to suppress plasma prolactin concentrations. Curlewis *et al* (1991) injected (i.m.) 2, 6 or 18 mg every 14 days of Parlodel long acting bromocriptine in Scottish Blackface ewes from 18th January until 25 July. Initially all doses significantly reduced plasma prolactin concentrations but later in the study (May and June) plasma prolactin concentrations were significantly suppressed only in the group receiving 18.0mg bromocriptine. Plasma prolactin concentrations increased in all groups during the treatment period.

In this experiment three dose rates were selected using the above information

with the aim of identifying a dose rate that would successfully suppress plasma prolactin concentrations to baseline levels for 14 days.

In Experiment 1, melatonin treatment from December resulted in an advance in the seasonal rise in plasma prolactin concentration. To provide information on whether this was due to the timing of the treatment or an artefact of the implant a further group received one dose of the melatonin treatment used in Experiment 1, i.e. one 18mg implant (Regulin).

MATERIALS AND METHODS

Animals: 16 Adult female feral or feral x Dairy type (Toggenburg or Saanen) goats with no previous history of hormone or light treatment, were penned indoors under the influence of natural photoperiod and temperature. The animals were fed *ad libitum* the complete pelleted diet used in Experiment 1 (AA6) based on barley and soya bean meal incorporated with 30% barley straw. Fresh water was available at all times. On the 18 March 1988, two weeks before treatment imposition, the animals were allocated to one of five treatment groups in a balanced manner by breed type and live weight.

Treatments: Treatment A (n=4) was a control group which received no treatment. In Treatment B (n=3) goats received one implant of 18mg of melatonin (Regulin Ltd, Australia) subcutaneously at the base of the ear on 1 April 1988. In Treatments C, D and E (n=3, each group) goats received 5, 15 or 25 mg of Parlodel L.A. (Sandoz Pharmaceuticals, Basle, Switzerland) in one i.m. injection on 1 April 1988.

The mean live weights (se) of each treatment group at the start of the experiment were 26.3 (2.60)kg, 37.3 (15.12)kg, 35.6 (11.6)kg, 38.3 (17.90)kg and 39.3 (19.65)kg respectively. The large range in live weight reflects the different

breed types used in each treatment group.

Sampling: On one occassion before treatment imposition and thereafter three times weekly for five consecutive weeks, 7ml blood samples were taken by jugular venepuncture into evacuated heparinised tubes. The samples were then centrifuged at 4deg C for 20 minutes and the plasma removed and stored at -20deg C. Prolactin concentrations were measured using the double antibody method of McNeilly and Andrew (1974) as described in Experiment 1. The intra and inter- assay coefficients of variation were <12.0 and 14.0 % respectively.

Statistical analysis: The natural logarithm of plasma prolactin concentration was compared between treatments by analysis of variance. The data were grouped for each week post treatment administration.

RESULTS

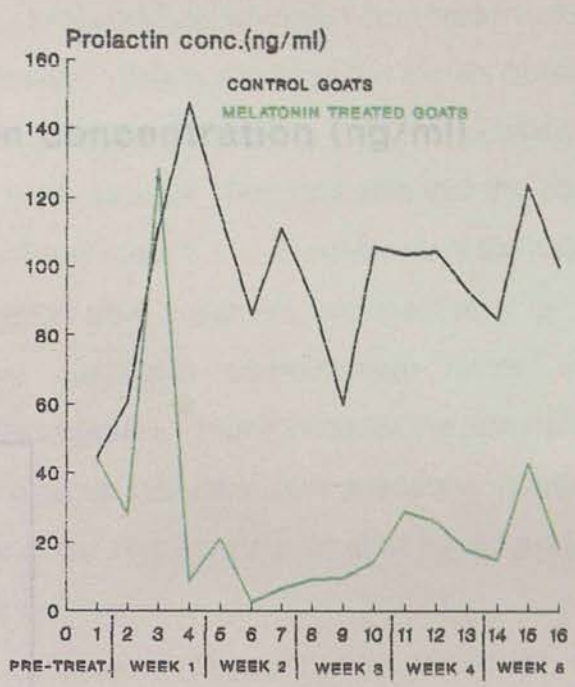
Mean weekly plasma prolactin concentrations are presented for each treatment in Table 1 and Figures 1a and 1b. Goats in Treatment B, which received the melatonin implant had significantly lower plasma prolactin concentrations on weeks 2 ($p<0.001$) 3 ($p<0.001$) and 4 ($p<0.05$) than the control animals. No significant differences were observed between the plasma prolactin concentrations of the control group and treatment groups C, D or E. There were significant differences between the plasma prolactin concentrations of the low and high dose bromocriptine-treated groups; Treatment B (5mg of bromocriptine) resulted in significantly higher plasma prolactin concentrations than those of treatment group E (25mg of bromocriptine) on weeks 1 and 2 ($p<0.05$). Figure 2 demonstrates a response curve of plasma prolactin concentration in week 1 for the three dose rates of bromocriptine.

Table 1. Mean plasma prolactin concentration (ng/ml) in goats treated with 18mg melatonin or 5, 15, or 25mg of Bromocriptine.

GROUP	SAMPLING TIMES					
	Pre-Treatment 1	Week 1 2 3 4	Week 2 5 6 7	Week 3 8 9 10	Week 4 11 12 13	Week 5 14 15 16
Treatment A: control (n=4)	44.60	60.96 108.90 133.19	102.51 77.63 100.36	94.63 60.05 105.7	94.76 104.49 93.25	84.18 120.47 93.36
Treatment B: melatonin (n=3)	44.29	28.20 116.29 8.64	21.29 2.44 6.45	9.10 9.00 14.54	25.45 26.34 19.87	15.11 40.84 18.44
Treatment C: Bromocriptine 5mg (n=3)	88.30	79.58 120.34 120.8	152.64 107.48 153.96	108.31 82.17 143.53	96.42 147.46 160.58	108.89 185.78 132.40
Treatment D: Bromocriptine 15mg (n=3)	121.55	29.28 60.57 105.69	42.19 44.92 106.05	104.6 62.44 99.21	68.63 166.69 116.61	38.89 119.72 48.52
Treatment E: Bromocriptine 25mg (n=3)	155.03	13.14 34.54 37.74	43.6 26.28 75.68	77.28 39.68 95.10	74.18 156.54 177.81	122.57 155.35 76.35
Mean s.e.d.	min-min 0.395 min-max 0.369					

Figures 1a and b. Plasma prolactin concentrations of animals treated with melatonin or 5, 15 or 25 mg of bromocriptine.

a) Plasma prolactin conc. of control and *melatonin treated goats*



b) Plasma prolactin conc. of control and *bromocriptine treated goats.*

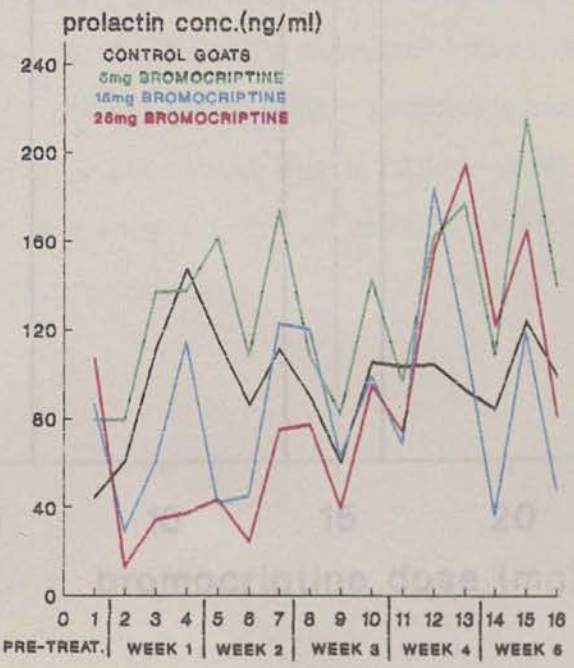
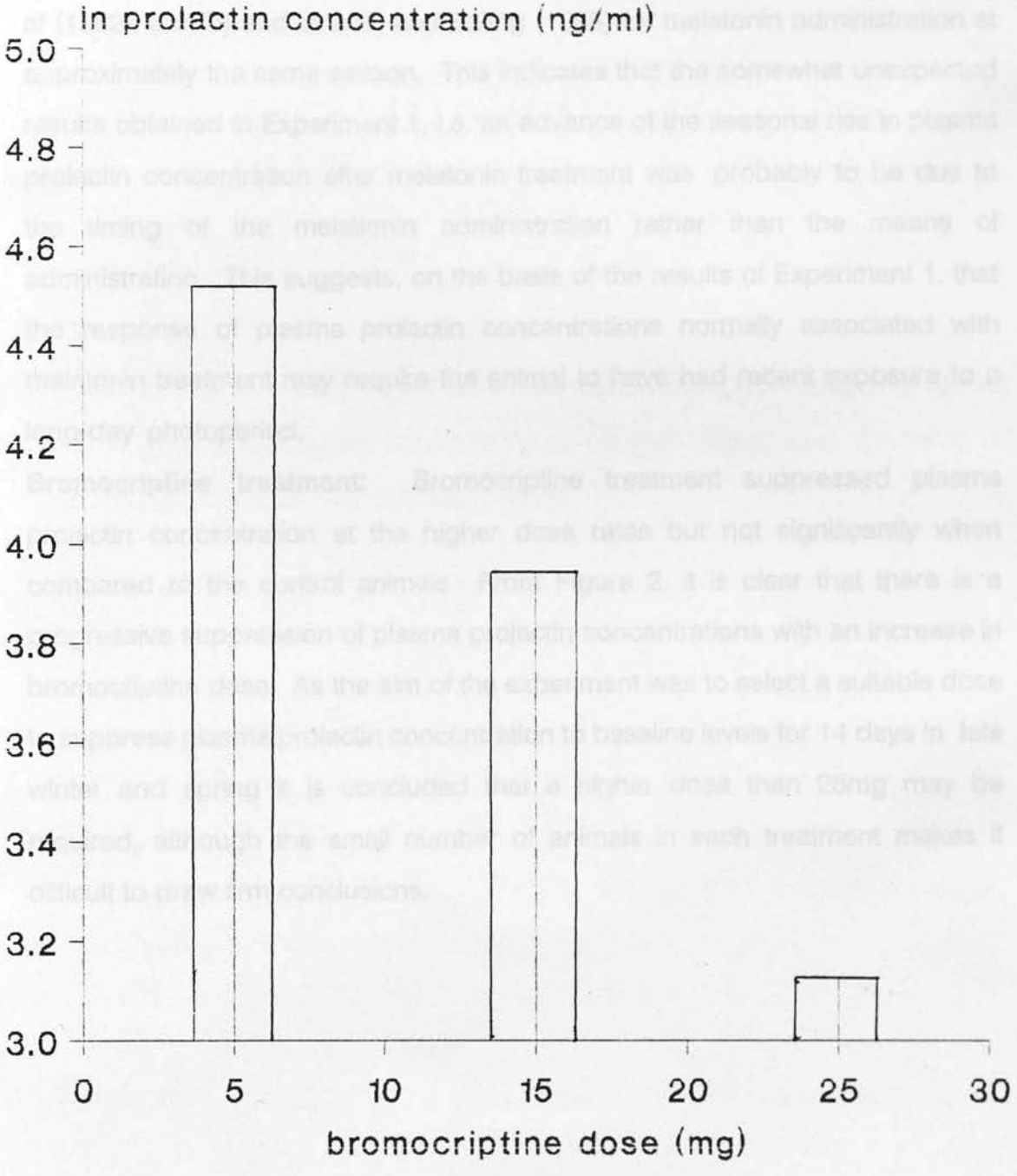


Figure 2. Mean plasma prolactin concentrations when treated with three doses of bromocriptine.



DISCUSSION

Melatonin treatment: Melatonin treatment in the spring significantly suppressed plasma prolactin concentration compared to the controls from one week after administration. This is similar to the results obtained by Kennaway *et al* (1982a and b) and Lincoln and Ebling (1985) for melatonin administration at approximately the same season. This indicates that the somewhat unexpected results obtained in Experiment 1, i.e. an advance of the seasonal rise in plasma prolactin concentration after melatonin treatment was probably to be due to the timing of the melationin administration rather than the means of administration. This suggests, on the basis of the results of Experiment 1, that the response of plasma prolactin concentrations normally associated with melatonin treatment may require the animal to have had recent exposure to a long-day photoperiod.

Bromocriptine treatment: Bromocriptine treatment suppressed plasma prolactin concentration at the higher dose rates but not significantly when compared to the control animals From Figure 2, it is clear that there is a progressive suppression of plasma prolactin concentrations with an increase in bromocriptine dose. As the aim of the experiment was to select a suitable dose to suppress plasma prolactin concentration to baseline levels for 14 days in late winter and spring it is concluded that a higher dose than 25mg may be required, although the small number of animals in each treatment makes it difficult to draw firm conclusions.

Chapter Four: The effect of advancing or suppressing the spring rise in plasma prolactin concentration on the timing and duration of the spring moult in the cashmere goat.

Experiment Three: The effect of advancing or suppressing the spring rise in plasma prolactin concentration on the timing and duration of the spring moult in the cashmere goat.

AIM

The aim of this study was to investigate the relationship between plasma prolactin concentration and changes in the hair follicle cycle and to identify methods of manupulating the timing of the spring moult to increase cashmere harvesting efficiency.

INTRODUCTION

In the first experiment of this study, 40 female adult and juvenile cashmere goats were implanted with melatonin (Regulin) on three occasions (December, February and April) with the expectation that the melatonin would suppress the spring rise in prolactin and delay moulting. The implanted adult females however, exhibited an earlier rise in prolactin than the control animals (6 weeks earlier). This was associated with an advance in the onset of moulting in these animals.

In the Djungarian hamster the annual pattern of pelage colour changes has been modified by the suppression or administration of prolactin (Duncan and Goldman, 1984). Suppression of endogenous prolactin by the dopamine agonist bromocriptine led to a delay in the spring moult to dark agouti pelage, while concommittant prolactin administration activated the moult.

To investigate the role of prolactin in controlling hair follicle activity it is necessary to demonstrate both the effect of suppressing plasma prolactin concentration when it is naturally rising and to increase plasma prolactin concentration when it is endogenously low. In this study the possibilities of manipulating the plasma

prolactin concentrations by either, using bromocriptine to suppress endogenous prolactin and/or exogenous ovine-prolactin to elevate plasma prolactin concentration are investigated and the inter-relationships between prolactin level, hair follicle activity and appetite are studied.

MATERIALS AND METHODS

Animals: Thirty eight adult female non-pregnant goats of cashmere type (three feral, sixteen 0.75 feral : 0.25 dairy type and ten 0.5 feral: 0.5 dairy type) were used. The dairy types were of the Toggenburg or Saanen breeds. The animals had had no history of hormone treatment or exposure to artificial photoperiod. At the start of the treatment imposition on 4 January 1989 the mean live weight (se) of the goats was 47.0 (8.12)kg.

Treatments: The animals were blocked by genotype and live weight and allocated to the following four treatments:

Treatment A (n=10) - untreated control animals

Treatment B (n=8) - animals received subcutaneous injections of 2mg ovine prolactin (NIDDK, NHPP, University of Maryland School of Medicine, Maryland, USA. NIDDK - OPRL - 19 (AFP-9221A) every 12 hours for 49 days (7 weeks) from 5 January 1989. The prolactin solution was prepared every 12 hours by dissolving 16.5mg of ovine-prolactin in 0.5 ml of 0.01M NaOH (BDH, AnalaR. Product No. 10252) and made up to 16.2ml with medical saline (Steriflex, Kendall Laboratories Ltd, Kendall, England) and aliquotted into eight 2ml syringes.

Treatment C (n=12) - Animals received intramuscular injections of 35mg long-acting bromocriptine (Parlodel L.A., Sandoz, Basle, Switzerland) every 14 days

for 119 days (17 weeks) from 5 January 1989.

Treatment D (n=8) - Animals received injections of both prolactin and bromocriptine at the same dose rates and times as for Treatments B and C, for 49 days from 5 January 1989.

Management: The animals were individually penned on a slatted floor indoors from 23 December 1988 until 24 May 1989 under natural photoperiod and temperature.

A concentrate pellet (Seafield Mill, Edinburgh) containing 24.4% maize gluten feed, 20% Distillers dark grains, 13.2% barley, 12% malt culms and 7.2% rape (DM content, 870g/kg, ME, 12.5 MJ/kgDM) was mixed equally with grass pellets (Seafield Mill, Edinburgh) (DM content 850g/kg, ME, 10.0 MJ/kgDM) and offered *ad libitum.* A refusal margin of 10% was allowed. Chopped hay and straw (50g) were offered twice daily and fresh water was available at all times.

Measurements

Voluntary food intake and live weights: The VFI was estimated daily as the difference between the amount of food offered and that refused. Weekly values, expressed as VFI per day and meaned over 7 days for each animal, were used in the statistical analysis. The animals were weighed to the nearest 0.5kg at the beginning of the experiment and at 7 and 18 weeks.

Blood sampling: Blood samples (10ml) were taken by jugular venepuncture into heparinised evacuated tubes. The samples were centrifuged at 2,500 rpm for 20 minutes. The plasma was removed with a syringe and stored in 3ml vials at -20deg C. Samples were taken twice weekly at 1100h from all animals on each treatment and at 0, 1, 2, 4, 6, 8, 10 and 12 hours post injection of prolactin in Treatments B and D on 3 occasions, viz at the beginning of the period of

treatment imposition (10 January 1989), at the mid-point of the imposition of treatments (31 January 1989) and at the end of the imposition of treatments (20 February 1989). The serial blood samples were taken from smaller numbers of animals per treatment. Animals were chosen at random from each group: Treatment A (n=4), Treatment B (n=4), Treatment C (n=4), and Treatment D (n=6). The same animals were used on each occasion. Prolactin concentrations in the plasma were determined using the double antibody radioimmunoassay described in Experiment 1. Intra- and inter-assay coefficients of variation were less than 11.6% and 13.8% respectively.

Fibre measurements: To estimate the cashmere content of the coat, 10cm patches of fibre were clipped from different sites on the mid-side position to the rear of the last rib, every 28 days. The patches were measured using calipers with arms $\sqrt{10}$cm apart and samples were taken using surgical clippers. The samples were scored for content of cashmere, in reference to the photographic scale established in Experiment 1. As in Experiment 1, the patches of fibre were presented to the operator in a random manner to eliminate any preemptive bias.

The stage of moulting was assessed visually, every four weeks from February to July and scored according to the number of fibres released when the hand was brought smoothly down the coat, i.e. 0=no fibres released, 1=few fibres released, and 2=numerous fibres released. Initially, the coat was ruffled to remove broken fibres. At the end of the administration of prolactin in Treatments B and D, the number of animals in each group showing moulting around the eyes and ears, and those with cashmere 'raised' above the skin surface were recorded (it was observed in Experiment 1 that just prior to the moult the cashmere in the coat appeared to rise above the skin surface and to be held within the guard hairs. This was termed 'raised' cashmere.) All visual

assessments were made by the same operator and verified by a second independent observer. Skin samples of about 0.5cm in diameter were removed by biopsy from the mid-side position at the same time and on the same location as the 10cm^2 patch of fibre. The samples were processed and analysed as in Experiment 1.

Statistical analyses: There were three distinct time periods. These were (1) a pretreatment period (weeks -2 to 0) when animals became accustomed to their new environment and diet, (2) treatment period 1, when animals in all treatment groups received their treatments (weeks 0 to 7), and (3) treatment period 2, when animals in only Treatments A and C continued to receive their treatments (weeks 8 to 19).

The mean daily VFI's were expressed per kilogram of metabolic live weight to remove the variation between animals associated with a large range in live weight. Differences between treatments over the experimental periods and weekly were investigated by Analysis of Variance (Genstat 5.2.1, Rothamstead Agricultural Station).

There was a considerable range in live-weight within each group due to the variety of breed types used in the experiment. The data were therefore analysed by examining the differences between live weight at the beginning of the experiment, at the end of Period 1 and at the end of Period 2, for each animal. These differences were compared by analysis of variance. The natural logarithm of prolactin + 1 was used in the statistical analysis so that an assumption of equal variance between treatments could be made. A regression of (logarithm of prolactin concentration +1) against time was fitted for each animal in each treatment period i.e. Periods 1 and 2. Mean log prolactin concentrations were adjusted so that the intercept value was equivalent to the mean prolactin concentration within that time period for that individual. Values

of mean prolactin concentration and the slope of the fitted line were obtained in this manner for each animal in each treatment period. Differences between treatments in the values of mean concentration and slope were compared independently, examining the differences both between groups within each treatment period and within groups across Periods 1 and 2. For both concentration and slope, graphs of residuals vs fitted values and residuals vs normal quantiles were drawn. These demonstrated equal scatter and a straight line relationship respectively, indicating that analysis of variance was appropriate for these data.

Cashmere content scores and visual assessments were expressed as the proportion of animals in each group that received a particular score. These data are also presented graphically to illustrate the change from winter to summer coat in each treatment group. To analyse statistically the data for the cashmere content scores of the 10 cm patches, the number of animals in each group that scored 4 or 5, i.e. indicative of winter coat, and the number that scored less than 4, i.e. indicative of moulting having taken place, were calculated, and treatment differences compared using a Chi-squared test (Minitab, Minitab Inc, USA). To test the visual assessments of moulting the data were subdivided into the proportions of animals that received a score of 2, i.e heavy moult, and those that received a score of less than 2, i.e. either no or only slight moulting.

After selection of the optimum serial sections, i.e. a section cut at the level of the sebaceous gland and showing clear follicle groups, the primary and secondary follicles were categorized into one of three types: anagen, catagen or telogen, according to their size, stain colouring and appearance. The total number of each type in each of primary and secondary follicles in each section were counted. Only complete groups of primary and secondary follicles with

accessory structures were counted. The proportion of primary and secondary follicles in each category was calculated for each animal. Treatment differences in these values were compared for each sampling date by analysis of variance.

RESULTS

Voluntary food intake.

The mean daily VFI's (expressed as g/kg w**0.75)for each week in the treatment periods compared to that of the pre-treatment period are presented in Table 1. The logn of the deviations are presented in Figure 1 and it is evident that the VFI's of those treatment groups receiving prolactin injections, i.e. Treatments B and D, were higher than those of Treatments A and C. This was confirmed by analysis of variance, with animals that received prolactin, i.e. Treatments B and D, having significantly higher VFIs than those on Treatments A and C ($p<0.01$). There was also a significant difference between Treatments A and D, i.e. bromocriptine-plus-prolactin treatment (Treatment D) had significantly higher VFIs than those of the control treatment ($p<0.01$). There were no significant differences in VFI between the other treatments. In all treatments there was a significant effect of time ($p<0.001$) with VFIs increasing over both experimental periods.

Live weights: The mean live weight of each treatment group at the beginning of the experiment and at the end of Periods 1 and 2 together with the live-weight gains for each period are presented in Table 2. There were no significant differences between treatments in live weight or live-weight gain in Periods 1 or 2. There was a significantly higher live-weight gain for all groups ($p<0.001$) in Period 2 than in Period 1.

Plasma prolactin concentrations: Mean plasma prolactin concentrations are presented for each treatment in Figure 2. As described above, a regression of plasma prolactin concentration against time was fitted to the data for each animal in Periods 1 and 2. The mean values of the intercept (i.e mean prolactin concentration) and slope are presented in Tables 3 and 4 respectively. From Table 3 it can be seen that Treatments B and D had significantly higher

Table 1 **Mean voluntary food intake g/kg lwt**0.75/day of each group on each week of the experiment**

GROUP	WEEK OF EXPERIMENT																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A: Control (n=10)	61.2	68.9	71.4	63.8	69.4	63.9	71.3	67.1	70.0	71.5	73.7	68.8	67.5	68.0	65.7	65.2	73.0	70.5	72.7
B: Prolactin (n=10)	58.9	52.8	67.7	64.3	73.0	72.6	79.1	75.2	81.5	70.3	78.5	78.2	81.2	74.4	69.3	70.2	76.8	77.2	81.3
C: Bromocriptine (n=10)	61.0	54.6	60.4	61.1	72.3	64.1	73.4	71.9	73.8	70.3	72.5	67.2	73.8	66.1	62.8	66.2	75.0	71.8	73.1
D: Bromocriptine plus Prolactin (n=8)	70.8	84.6	85.8	71.3	82.7	87.0	81.1	83.3	86.3	82.8	77.0	83.6	80.0	75.9	81.3	74.0	80.0	82.4	86.8
Mean s.e.d.	min-max 8.99 max-max 8.48																		

Figure 1. Mean daily VFIs (log g/kg lwt0.75/day) for each group over both treatment periods.**

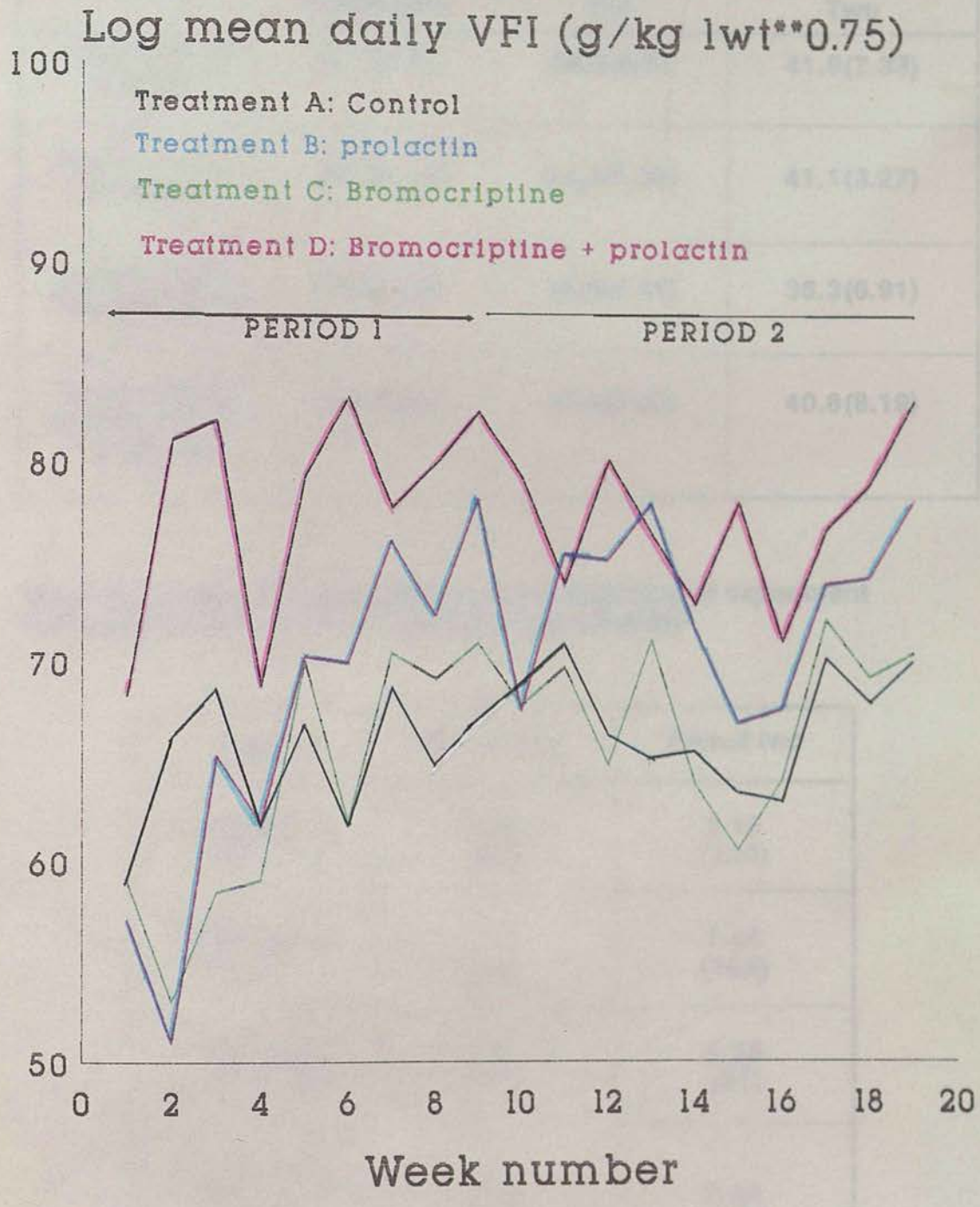


Table 2a. Mean live weight (s.e.) kg of each treatment group at three stages of the experiment.

Group	Beginning of Experiment	End of Period one	End of Period Two
Treatment A: Control	33.1(6.17)	35.2(6.31	41.9(7.33)
Treatment B: Prolactin	32.1(6.34)	34.3(5.36)	41.1(3.27)
Treatment C: Bromocriptine	29.9(3.00)	30.6(5.45)	36.3(6.91)
Treatment D: Bromocriptine + Prolactin	32.4(6.01)	34.0(7.23)	40.8(8.19)

Table 2b. Mean live-weight gain (kg) from beginning of experiment to the end of Periods 1 and 2 (g/day in parenthesis).

Group	Period one	Period two
Treatment A: Control	2.35 (48)	9.10 (130)
Treatment B: Prolactin	1.75 (36)	7.64 (109)
Treatment C: Bromocriptine	0.71 (15)	6.38 (91)
Treatment D: Bromocriptine + Prolactin	1.56 (32)	8.44 (120)
s.e.d. min-min min-max	1.280 1.169	1.678 1.532

Figure 2. Mean prolactin concentrations.

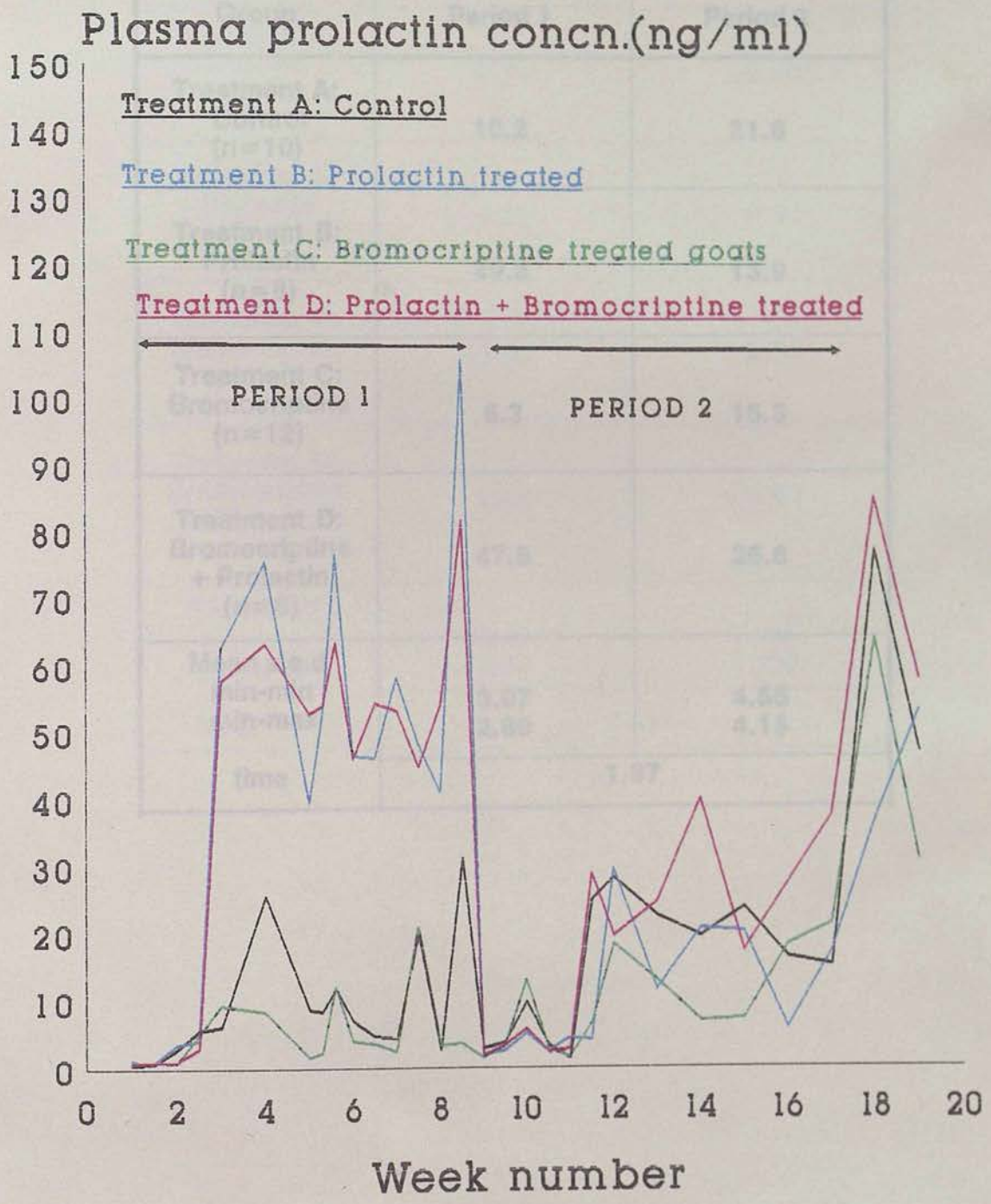


Table 3. Mean plasma prolactin concentration (ng/ml) calculated from regressions of logn plasma prolactin concentration +1.

Group	Period 1	Period 2
Treatment A: Control (n=10)	10.2	21.9
Treatment B: Prolactin (n=8)	49.3	13.9
Treatment C: Bromocriptine (n=12)	6.3	15.3
Treatment D: Bromocriptine + Prolactin (n=8)	47.5	26.6
Mean s.e.d min-min min-max	3.07 2.80	4.55 4.15
time	1.87	

Table 4. Mean slope of fitted regressions of logn plasma prolactin concentration +1 on time in Periods 1 and 2.

Group	Period 1	Period 2
Treatment A: Control (n=10)	0.70	3.60
Treatment B: Prolactin (n=8)	3.38	2.95
Treatment C: Bromocriptine (n=12)	0.08	2.81
Treatment D: Bromocriptine + Prolactin (n=8)	2.48	5.00
Mean s.e.d		
min-min	1.248	1.440
min-max	1.139	1.320
time	0.614	

($p<0.001$) mean plasma prolactin concentrations than Treatments A or C in Period 1 whereas in Period 2, Treatment D had a significantly higher mean prolactin concentration than Treatments B and C but was not significantly different from those of Treatment A. Plasma prolactin concentrations for Treatment A were also significantly greater than those for Treatments B and C ($p<0.01$ and $p<0.05$ respectively).

The change in mean plasma prolactin concentrations between the two time periods was investigated for each treatment. Treatments A and C showed a significant increase from Period 1 to 2 ($p<0.001$ and $p<0.01$ respectively), whereas Treatments B and D demonstrated a significant decrease ($p<0.001$) (Table 3).

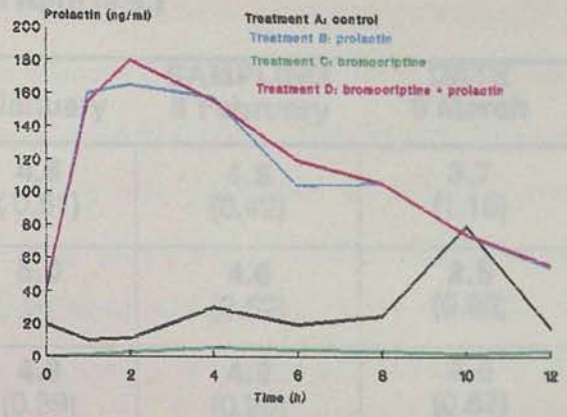
The mean slopes of the fitted lines are presented in Table 4. In Period 1, there were no significant differences between Treatments A, B and D in slope. Treatment C had a significantly greater slope compared to Treatments A, B and D ($p<0.01$). In Period 2 there were no significant differences between treatments. When the change between time periods was investigated there was a significant increase in slope in Treatments A, B and D ($p<0.001$) from Period 1 to period 2 and no significant difference in Treatment B (Table 4).

The mean prolactin concentrations of the animals in each treatment selected for the serial blood sampling are presented in Figure 3. At all three dates for the first 8 hours post injection, Treatments B and D had significantly greater plasma prolactin concentrations ($p<0.001$). At 10 and 12 hours post injection on all three dates the plasma prolactin concentrations of Treatments B and D had decreased to levels that were not significantly different from those of Treatments A and C.

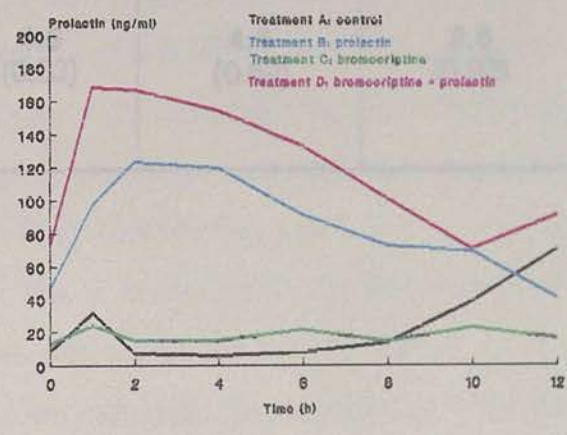
Cashmere content scores: Mean cashmere content scores, calculated monthly for each treatment group, are presented in Table 5. A cashmere

Figure 3. Plasma prolactin concentration of animals from each group sampled for up to 12 hours post treatment injection.

a. 10.01.89



b. 31.01.89



c. 20.02.89

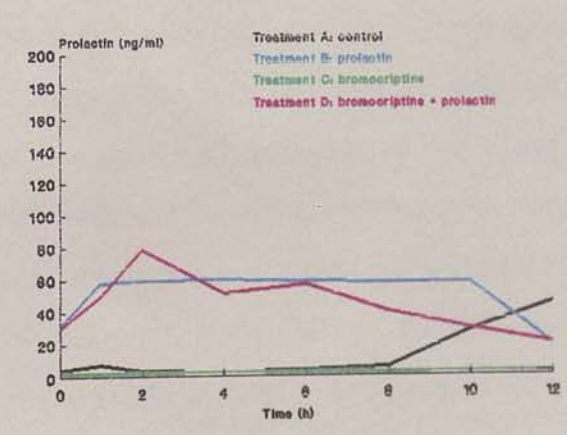


Table 5. Mean cashmere content score of each group in January, February, March and April (s.e.)

GROUP	SAMPLING DATE 6 January	8 February	9 March	7 April
Treatment A: Control	4.6 (0.51)	4.8 (0.42)	3.7 (1.16)	3.1 (1.20)
Treatment B: Prolactin	5.0 (-)	4.6 (0.52)	2.5 (0.93)	2.3 (0.49)
Treatment C: Bromocriptine	4.8 (0.39)	4.9 (0.29)	4.5 (0.67)	4.3 (0.97)
Treatment D: Bromocriptine + prolactin	4.5 (0.53)	4.6 (0.52)	2.6 (0.92)	2.4 (0.56)

content score of less than 4 is indicative of the moult from winter coat to summer coat. In January and February there were no significant differences between the treatments in cashmere content score. In March, Treatments A and C had significantly higher cashmere content scores than Treatments B and D ($p<0.001$). In April, Treatment C had a significantly higher cashmere content score than the other treatments ($p<0.01$). The proportion of animals in each treatment that scored 4 or 5 was calculated each month and is presented graphically in Figure 4. It is clearly demonstrated that there were differences between treatments in the timing of the moult. In the control group there was a steady decrease in the proportion of animal with a winter coat from February until April when 0.30 of the animals scored 4 or 5. In comparison Treatments B and D demonstrated a much more rapid loss of winter coat; in Treatment B none of the animals scored 4 or 5 in March, and in Treatment D only 0.13 of the animal had such scores. In contrast, the bromocriptine-treated group, treatment C, showed an only very gradual reduction in score, with 0.92 of the animals in March and 0.83 in April scoring 4 or 5.

Visual assessments: Visual assessments on the degree of moulting were made every four weeks starting at the end of Period 1. The proportion of animals that scored 2 for moulting (representative of undergoing a heavy moult) are presented in Figure 5. In the control group between 0.50 and 0.70 of the animals were moulting heavily at all four sampling dates, indicating asynchrony within the group. The prolactin and prolactin-plus-bromocriptine treated groups showed a more rapid decrease in the proportion of animals moulting from the end of treatment Period 1, indicating an advance in moulting compared to the controls and a more closely synchronised moult within both groups. In contrast, in Treatment C there were no animals moulting in March or April with a gradual increase in the proportion in May and June.

Figure 4. Proportion of clipped patches from each group that scored 4 or 5.

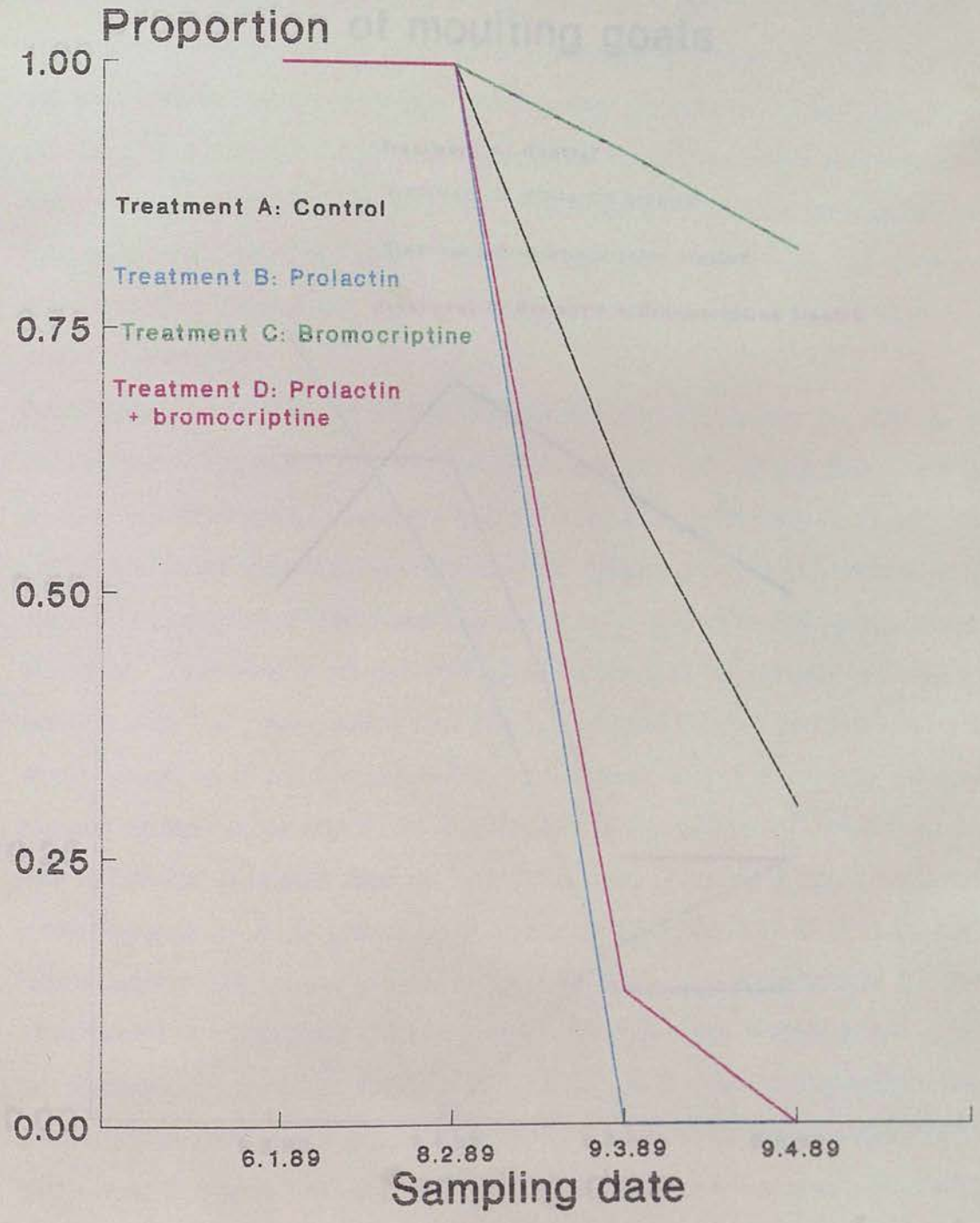
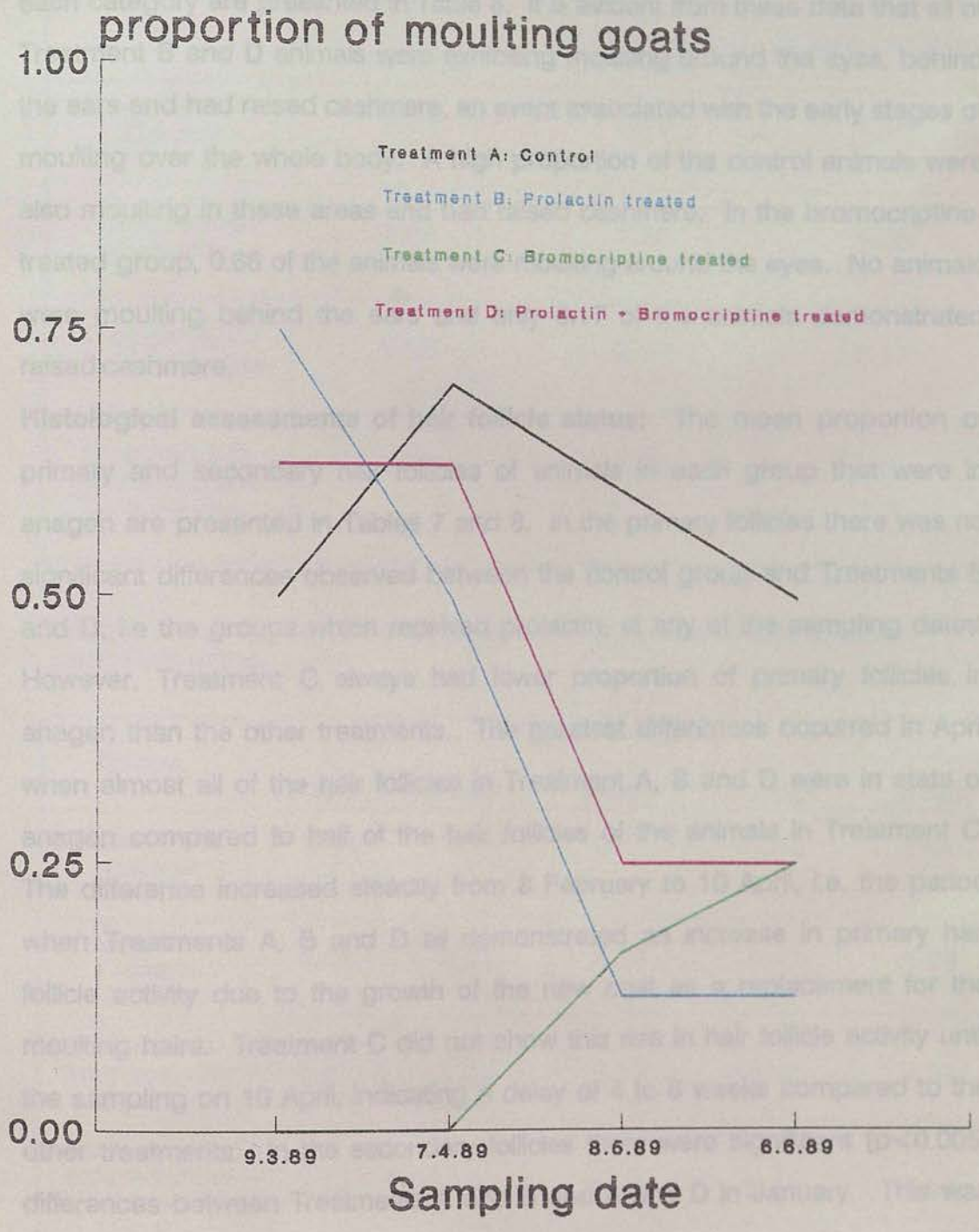


Figure 5. Proportion of moulting animals in each group.



At the end of treatment Period 1 the presence of moulting around the eyes and behind the ears and the occurrence of 'raised' cashmere were assessed visually for each animal. The proportions in each group that were scored for each category are presented in Table 6. It is evident from these data that all of Treatment B and D animals were exhibiting moulting around the eyes, behind the ears and had raised cashmere, an event associated with the early stages of moulting over the whole body. A high proportion of the control animals were also moulting in these areas and had raised cashmere. In the bromocriptine-treated group, 0.66 of the animals were moulting around the eyes. No animals were moulting behind the ears and only 0.17 of the animals demonstrated raised cashmere.

Histological assessments of hair follicle status: The mean proportion of primary and secondary hair follicles of animals in each group that were in anagen are presented in Tables 7 and 8. In the primary follicles there was no significant differences observed between the control group and Treatments B and D, i.e the groups which received prolactin, at any of the sampling dates. However, Treatment C always had lower proportion of primary follicles in anagen than the other treatments. The greatest differences occurred in April when almost all of the hair follicles in Treatment A, B and D were in state of anagen compared to half of the hair follicles of the animals in Treatment C. The difference increased steadily from 8 February to 10 April, i.e. the period when Treatments A, B and D all demonstrated an increase in primary hair follicle activity due to the growth of the new coat as a replacement for the moulting hairs. Treatment C did not show this rise in hair follicle activity until the sampling on 10 April, indicating a delay of 4 to 8 weeks compared to the other treatments. In the secondary follicles there were significant ($p<0.005$) differences between Treatments A and B and A and D in January. This was

Table 6. Proportion of animals exhibiting moulting around the eyes, behind the ears and 'raised' cashmere at the end of treatment Period 1.

Group	Moulting around the eyes	Moulting behind the ears	Raised cashmere
Treatment A: Control	0.90	0.70	0.90
Treatment B: Prolactin	1.00	1.00	1.00
Treatment C: Bromocriptine	0.66	0.00	0.17
Treatment D: Bromocriptine + Prolactin	1.00	1.00	1.00

Table 7. Mean proportion of primary follicles in anagen (se) in each treatment group.

Group	Date of sampling				
	10 Jan	8 Feb	9 Mar	10 Apr	8 May
Treatment A: Control (n=10)	0.08	0.10	0.51	0.93	1.00
Treatment B: Prolactin (n=10)	0.14	0.20	0.50	0.83	0.99
Treatment C: Bromocriptine (n=10)	0.07	0.05	0.13	0.50	0.94
Treatment D: Bromocriptine + Prolactin (n=8)	0.05	0.20	0.62	0.86	0.99
mean s.e.d.					
min-max	0.050	0.071	0.133	0.117	0.030
max-max	0.045	0.065	0.121	0.107	0.027

Table 8. Mean proportion of secondary follicles in anagen in each treatment group.

Group	Date of sampling				
	10 Jan	8 Feb	9 Mar	10 Apr	3 May
Treatment A: Control (n=10)	0.02	0.13	0.53	0.95	0.97
Treatment B: Prolactin (n=10)	0.16	0.33	0.66	0.68	0.97
Treatment C: Bromocriptine (n=10)	0.02	0.03	0.09	0.49	0.96
Treatment D: Bromocriptine + Prolactin (n=8)	0.19	0.18	0.67	0.77	0.99
Mean s.e.d	0.071	0.072	0.133	0.148	0.021
min-max	0.065	0.066	0.121	1.135	0.019
max-max					

due to the control animals in Treatment A having a very low proportion of follicles in anagen, 0.019. The proportion of secondary follicles in anagen for Treatment A was also significantly lower than that for Treatment B in February ($p<0.01$). Thereafter there were no significant differences between these treatments. Treatment C was significantly ($p<0.05$) lower than Treatments A, B and D in the proportion of secondary follicles in anagen for February, March, and April with the greatest difference occurring in March ($p<0.001$). This is in contrast to the results for the primary hair follicles, where the greatest differences occurred in April.

DISCUSSION.

This experiment required the manipulation of plasma prolactin concentrations in Treatments B, C and D. Figure 2 clearly demonstrates that this was achieved. In Treatments B and D plasma prolactin concentrations were elevated during the period of treatment administration i.e. Period 1. In Period 2 after prolactin administration had ceased, initial plasma prolactin concentrations, which were now from only endogenous sources, were low. As Period 2 progressed, plasma prolactin concentrations in all groups increased, with Treatments A and D having significantly ($p<0.05$) higher mean concentrations than Treatments B or C. However, no differences were observed in the slopes of the regression of plasma prolactin concentration on time indicating that in all treatments plasma prolactin concentrations increased at the same rate over Period 2. It is unclear why Treatment D (prolactin-plus-bromocriptine) should have a significantly higher mean plasma prolactin concentration than Treatment B (prolactin) in Period 2 as both treatments received the same amounts of exogenous prolactin. The different response to cessation of treatment imposition indicates that the difference in endogenous control of prolactin synthesis and release, via the presence of absence of bromocriptine combined with prolactin treatment, resulted in significantly different post treatment plasma prolactin concentrations. The increase in plasma prolactin concentration observed in Treatments A, B and D is likely to be due to the seasonal effect associated with increasing daylength (Ravault, 1976; Buttle, 1974, and Lincoln, McNeilly and Cameron, 1978). The increase in circulating prolactin concentration observed in Treatment C at the end of Period 2 is not unexpected when the results of other long term bromocriptine treatment impositions are considered. Curlewis *et al* (1988) treated red deer hinds with long term bromocriptine treatment from mid-

winter. In the first three months of treatment prolactin was suppressed to non-detectable levels but gradually increased over the subsequent five months, although remaining significantly lower than the control values. In a similar study on Scottish Blackface ewes (Curlewis *et al,* 1991) animals were treated with 0, 2.0mg, 6.0mg or 18.0mg of bromocriptine from 18 January to 23 July. Bromocriptine suppressed plasma prolactin concentration in all groups from January until April, but in May and June plasma prolactin concentrations were suppressed only in the group given the highest dose, although even in this group plasma prolactin concentrations were increasing. When animals receiving bromocriptine were challenged with a further 10mg of bromocriptine, plasma prolactin concentrations were suppressed. It was suggested by Curlewis *et al* (1991) that decreased sensitivity to bromocriptine in animals already receiving bromocriptine was due to the seasonal decrease in dopamine. In winter, bromocriptine would summate with endogenous dopamine leading to suppression of plasma prolactin concentrations to non-detectable levels. In summer, levels of dopamine would be lower to permit the seasonal increase in plasma prolactin concentrations and a greater dose of bromocriptine would be required to adequately suppress plasma prolactin. However, the release of prolactin from the anterior pituitary is not a simply regulated process, but a complex series of events which includes synthesis, storage, release and degradation (Dannies, 1982). Blask and Orstead (1986) reported that dopamine inhibits the release of prolactin from the anterior pituitary gland in the male Syrian hamster exposed to a long photoperiod but not the synthesis of prolactin. They concluded that dopamine was an important regulator of short term prolactin release but not synthesis under a long-day photoperiod. This is in contrast to pineal-mediated prolactin suppression expressed during long-term light deprivation which results in synthesis, storage

and release of prolactin being inhibited in male and female hamsters (Black, Leadem, Orstead and Larsen, 1986). To test whether the increase in prolactin observed after prolonged bromocriptine treatment is due to the seasonal decrease in dopamine levels it would be necessary to give increasing doses of bromocriptine over the experimental period.

One other possible explanation is that prolonged treatment may lead to dopamine receptor down regulation at the lactotroph. However, when bromocriptine treatment was continued until November, when plasma prolactin concentrations have normally declined, bromocriptine was again effective in suppressing plasma prolactin concentrations, suggesting that refractoriness to bromocriptine does not occur with long term bromocriptine treatment (Curlewis et al, !988, sheep). In light of this information, we can reconsider why Treatment D had a significantly higher mean plasma prolactin concentration ($p<0.001$) in Period 2 when compared to Treatment B. Treatment D animals were subjected to high rates of turnover of dopamine which stopped at the end of bromocriptine treatment and which could have led to down regulation of the dopamine receptors and thus decreased sensitivity to natural levels of dopamine after treatment. This could result in a greater increase in the synthesis and release of prolactin. However, the fact that the prolactin concentrations in Treatment D were not significantly higher than those in the control group, implies that it is Treatment B, the prolactin-treated group, that has decreased sensitivity to increasing daylength and the stimulus to synthesis and release prolactin.

Plasma prolactin concentration was also measured at 0, 1, 2, 4, 6, 8, 10 and 12 hours post-injection. There was a rapid increase in plasma prolactin-concentration in Treatments B and D within one hour of the injection of prolactin. Concentration decreased over the 12 hour sampling period but

remained higher than Treatment A and D for almost all the twelve hours post sampling. In the control animals a peak was observed at 10 hours post injection time, the onset of darkness. A similar observation was also recorded by Muduuli *et al* (1979) in the male pygmy goat which, they suggest, is supporting evidence that a hypothalamic-hypophyseal photosensitive phase is present during the 24 hours cycle as previously suggested in the ram (Ravault and Ortavant, 1977).

As Period 1 progressed, plasma prolactin concentrations appeared to decrease. On sample date 2, this is most evident in Treatment B, suggesting that in Treatment D the agonistic effects of bromocriptine led to a slower degradation of exogenous prolactin. However, it could also be related to the amount of bromocriptine present in the circulation. The three serial blood sampling dates occurred on days 7, 13, and 5 of bromocriptine treatment and on the second sample date (31 January) it can be seen that the plasma prolactin concentrations of Treatment C (bromocriptine only treatment) are higher than on 20 February, suggesting that the effect of the bromocriptine injection on plasma prolactin concentrations may be greater immediately following injection.

Voluntary food intakes: Treatments B and D, i.e. those animals that received prolactin or prolactin-plus-bromocriptine had significantly ($p<0.005$) higher VFI's than the control or bromocriptine treatments. No difference was observed between Treatment C, the bromocriptine treated group, and the control treatment. This is in contrast to the results of Eisemann *et al* (1984). They maintained two groups of sheep under two photoperiods: long days (16L:8D), and short days (8L:16D). In the long-day group, half of the animals received bromocriptine. The bromocriptine-treated sheep had significantly lower cumulative live-weight gains and VFIs. In the short-day group in which half of

the animals were treated with prolactin, no significant differences were observed in cumulative live-weight gain or VFI between the prolactin-treated and control groups. Effects of bromocriptine were also observed by Curlewis *et al* (1988) who found that treatment of red deer hinds from mid-winter with a long-acting formulation of bromocriptine resulted in reduced VFI, and in delays in the onset of anaestrus and the spring moult, suggesting a possible role from prolactin in regulating seasonal appetite changes in red deer. In the control animals in this experiment, a seasonal increase in appetite over the time course of the experiment was not evident. This observation was also noted in Experiment 1. As discussed previously, there is a lack of evidence on the presence of seasonal appetite in the goat. The absence of a seasonal increase in appetite over the experimental period may explain why the bromocriptine-treated animals with suppressed plasma prolactin concentrations did not differ significantly from the controls in VFI. A greater elevation of VFI in Treatments B and D with time implies a small seasonal fluctuation in VFI, regulated by prolactin. Curlewis et al, (1988) pointed out in their experiment that a direct effect of bromocriptine not mediated by the dopaminergic pathway could not be ruled out. In this experiment, a concomitant prolactin and bromocriptine treatment was included so that any effects of bromocriptine that are overridden by prolactin can be attributed to the suppression of prolactin rather than other side effects of bromocriptine associated with a dopaminergic pathway.

Live weight: Although there were no significant differences in live-weight gain between the treatments, the results generally follow those for VFI. For example, the live-weight gains of the prolactin treatments (Treatments, B and D) show a trend of being higher than for the bromocriptine treatment (Treatments C) and the live-weight gains were higher in Period 2 than in Period 1 in a similar manner to VFI. As discussed in Experiment 1, if any of the treatments are to be

proposed as methods of controlling fibre harvesting in goats, it is essential to ensure that there is no great live-weight loss as a result of any treatments. As no significant differences were observed between the treatments, and as all treatments resulted in live-weight gains it can be concluded that such a criterion was met by the treatments imposed in this experiment.

Cashmere content scores: The rate of progress of the spring moult can be determined, from the change in cashmere content scores and the rate of decrease from scores 4 or 5, representative of winter type coat, to scores of 1 or 2, where no cashmere is present, Statistical analysis of the scores demonstrated that the principal effects of the treatments on shedding occurred in March and April. In the control treatment there was a steady decrease in score from February until May, indicating that moulting was spread over a four month period. In Treatments B and D there was a much more rapid decrease in score, indicating not only an advance in the attainment of the summer coat but also a reduction in the duration of moulting within each group. This could be due to greater synchronisation between animals within the group and greater synchronisation between hair follicles within an individual.

The results from Treatment C indicate that the suppression of plasma prolactin concentration prevents or delays the moult. This result, taken together with the those for Treatments B and D where an increase in plasma prolactin concentration advanced the moult, clearly demonstrates a role for prolactin in the control of shedding. Visual assessments were also made on the degree of moulting. These provide supporting information to the cashmere content scores and prevents a potential bias in scoring clipped patches where some of the cashmere loss may be due to the breakage of hairs rather than moulting. It is obvious from Figure 8 that the treatments demonstrate a very similar pattern

of moulting to that described by the cashmere content scores. Treatments B and D demonstrate exactly the same proportion of animals moulting at each sampling date. By May the majority of animals in Treatments B and D had completed moulting. In contrast, in Treatment A there was a slower decline in the proportion of animals moulting which, as was concluded from the cashmere content scores, indicates a less condensed moult. In treatment C, no animals were moulting in March or April and moulting was significantly later than the other treatments. The increase in the proportion moulting before the end of bromocriptine treatment coincided with the reduction in the effectiveness of bromocriptine to suppress plasma prolactin concentration.

Histological status of primary and secondary hair follicles.

The proportion of primary follicles in the state of anagen i.e. actively growing, represents of the proportion of follicles that have completed a hair follicle cycle, have moulted the old fibre and are growing its replacement. All hair follicles undergo intermittent periods of growth when a new hair is produced, regression of the mature fibre to a resting state, quiescence and finally regeneration of a new fibre normally associated with loss of the mature fibre (Johnson, 1977; Spearman, 1977). The direct stimulus for a follicle to shed its fibre and initiate growth has not yet been identified. In this experiment the synchronisation of the follicles over the mid-region of the body, rather than individual follicles within different areas, were examined and this allowed one facet of synchronisation to be investigated. No differences were observed between Treatment A, B and D in the proportion of primary follicles in anagen although Treatments B and D did have a slightly higher proportion of follicles in activity than did the controls. Treatment C animals had a significantly lower proportion of follicles in anagen compared to those in Treatments B and D in

February ($p<0.05$) and compared to all treatments in March (<0.01) and April ($p<0.001$). These results demonstrate the suppression of moulting in Treatment C at a time when moulting was proceeding rapidly in the other treatments.

The differences in secondary follicle activity present a similar pattern to the primary follicles. However, differences between treatments occurred earlier, in January, indicating changes occurring in the secondary follicles in advance of the primary follicles. Also, Treatments B and D had higher proportions of active secondary follicles than the controls in January and February. This evidence, from the histological examination of the hair follicles, strongly supports the conclusions that have already been drawn from the cashmere content scores and visual assessment data i.e. that the premature elevation of plasma prolactin concentration advanced the onset of moulting and hair follicle activity. It is concluded that in the cashmere goat, the seasonal spring rise in plasma prolactin concentration is necessary to stimulate individual hair follicle cycles and thereby regulate the timing of the moult from a winter to a summer type coat. High plasma prolactin concentrations may be involved in reactivating the hair follicles either directly or via another messenger, but elevation of plasma prolactin concentration led to a more synchronised moult within the treatment groups. This greater sensitivity of the hair follicles to the prolactin signal may be due to the timing of the treatment (i.e. when endogenous prolactin is very low) and require further investigation.

Chapter Five: Manipulation of plasma prolactin concentration by either exogenous ovine prolactin, bromocriptine or sulpiride administration to induce and synchronise the spring moult in a group of animals.

Experiment Four: Manipulation of plasma prolactin concentration by either exogenous ovine prolactin, bromocriptine or sulpiride administration to induce and synchronise the spring moult in a group of animals.

INTRODUCTION

It was concluded from the results of Experiment 3 that, if a rise in plasma prolactin concentration is required to initiate the moult in an individual and has the effect of synchronising the moult within a group, a period of suppression, followed by a rapid increase in plasma prolactin concentration would be likely to induce the moult at a chosen time and also to achieve synchrony of moulting within a group. This would have important advantages for harvesting cashmere by enabling combing of the cashmere to be carried out on all animals at the same time.

To investigate this hypothesis plasma prolactin concentrations were manipulated to create treatments where animals either experienced a period of suppression of plasma prolactin concentration followed by a rapid increase in concentration, a more prolonged period of suppression, or a natural photoperiodically controlled rise in plasma prolactin concentration. To achieve this, one method of suppression of plasma prolactin and two methods of increasing plasma prolactin concentration were employed. Bromocriptine, which was used in Experiments 2 and 3 was employed to suppress plasma prolactin concentration. As discussed in Experiment 2, bromocriptine is a dopamine agonist and increases the rate of turnover and synthesis of dopamine (Blask and Orstead, 1986), inhibiting the release of prolactin from the pituitary. 'Sulpiride' was used as one method to stimulate prolactin secretion. 'Sulpiride' is a dopamine antagonist, acting directly on the lactotrophs (McLeod and Robyn, 1977) by the suppression of dopamine (Aidera *et al*, 1981).

'Sulpiride' has been demonstrated to elevate plasma prolactin concentrations in humans (Robyn, 1976) and monkeys (mangabey and patas monkey, Aidera *et al,* 1981). The other method used to increase plasma prolactin concentrations was the administration of exogenous ovine-prolactin. This had been successfully used in Experiment 3, to elevate plasma prolactin concentrations.

MATERIALS AND METHODS

Animals: Forty castrate 'cashmere' goats consisting of 10 feral, 24 feral x dairy type and 6 Icelandic x feral were used. The dairy types were of the Saanen or Toggenburg breeds. The goats were all 3 years of age and had no previous history of hormone treatment or exposure to artificial photoperiod. The mean live weight (se) of the goats on 22 December 1989 at the start of the experiment was 47.0 (+/- 8.15kg).

Treatments: The animals were balanced for genotype and live weight and allocated to one of four treatments.

Treatment A: (n=10)- untreated control animals.

Treatment B: (n=10)- animals received i.m. injections of 50mg of a long acting formulation of bromocriptine (Parlodel L.A. Sandoz Pharmaceuticals, Horsforth, Leeds, UK) every 14 days from 22 December 1989 until 24 May 1990.

Treatment C: (n=10)- animals received i.m. injections of 50mg of bromocriptine (as in Treatment B) every 14 days from 22 December 1989 until 24 May 1990 and s.c. injections of ovine-prolactin (NIDDK, NHPP, University of Maryland, School of Medicine, Maryland USA) at a dose rate of 2mg/12 hours from 1 April 1990 until 30 May 1990.

Treatment D: (n=10)- animals received i.m. injections of 50mg of bromocriptine (as in treatment B) from 22 December 1989 until 15 March 1990 followed by i.m.

injections of 'sulpiride' at a dose rate of 10mg/12 hours from 1 April 1990 until 30 May 1990.

The prolactin solution injected was prepared as in Experiment 3 by dissolving 20.01mg in 0.5ml of 0.1M NaOH (Sigma Chemical Co Ltd, Dorset, UK) and making up to 21 ml with medical saline (Steriflex, Kendall Laboratories, Kendall, England) and aliquoting into ten 2ml syringes. 0.101g of 'sulpiride' (N-1-[Ethylpyrrolidin-2-ylmethyl]-2-methoxy-5-sulfamoylbenzamide, Sigma Chemical Co Ltd, Dorset, UK) was suspended in 5.1ml of Arachid oil (BP Products, UK) and aliquoted into ten 0.5ml syringes. Each solution was freshly prepared every 12 hours just prior to injection.

Management: The animals were individually penned on a slatted floor indoors. Artificial lighting was programmed to emulate natural photoperiod at latitude 55.9 deg N. The times of sunrise and sunset were provided by the Royal Observatory, Edinburgh. As in Experiment 3, a concentrate pellet (Seafield Mill, Edinburgh) containing 24.4% maize gluten feed, 20% Distillers dark grains, 13.2% barley, 12% malt culms and 7.2% rape. (DM content, 870g/kg, ME, 12.5 MJ/kgDM) was mixed equally with grass pellets (Seafield Mill, Edinburgh) (DM content 850g/kg, ME, 10.0 MJ/kgDM) and offered *ad libitum*. A refusal margin of 10% was allowed. Chopped hay and straw (50g) were offered twice daily and fresh water was available at all times.

Measurements and sampling.

Voluntary food intake and live weights: The VFI was measured daily as the difference between the amount of food offered minus that refused. Mean daily values, the average over 7 days for each animal, were used in the statistical analysis. The animals were weighed to the nearest 0.5kg on 22 December 1989, 2 April 1990 and 31 May 1990.

Blood sampling: Blood samples (10ml) were taken by jugular venepuncture

into heparinised evacuated tubes. The samples were centrifuged at 2,500 rpm for 20 minutes. The plasma was removed with a syringe and stored in 3ml vials at -20deg C. Samples were taken weekly at 1100h and on three occasions (4 April, 1 May and 29 May 1990) at 0, 1, 2, 4, 6, 8, 10 and 12 hours post prolactin or 'sulpiride' injection. Prolactin concentrations were determined using the double antibody radioimmunoassay described in Experiment 1. Intra- and inter- assay coefficients of variation were less than 11.4% and 13.8% respectively.

Fibre measurements: On three occasions, 3 March, 10 April and 18 May 1990, 10cm^2 areas of fibre were measured on the midside position using callipers with arms $\sqrt{10}$cm apart and clipped using surgical clippers (Oster clippers). At the end of the experiment the samples were scored for the content of cashmere according to the reference scale established in Experiment 1. The samples were presented to the operator in a random manner to eliminate any pre-emptive bias. On 25 May 4 cm^2 patches were measured using callipers with arms 2cm apart and clipped using surgical clippers. Patches were clipped from five designated areas on the animal, the face (jowl), collar, belly, topline and thigh. The samples were hand separated into cashmere and hair with forceps. Each component was weighed and the proportion of cashmere in the sample by weight was calculated.

Visual assessments: The degree of moulting was visually assessed fortnightly and scored according to the number of fibres released when the hand was brought smoothly down the coat i.e. 0=no fibres, 1=few fibres and 2=numerous fibres. Initially the coat was ruffled to remove any broken fibres. As in Experiment 3, moulting around the eyes ears and the presence of 'raised' cashmere were also recorded.

Horn growth: Thirty four of the goats were horned. On 2 April a mark was

made on the outside ridge of the horn 50mm from the base using a hack saw blade and shortened ruler. Thereafter, the distance between the mark and the base of the horn was measured fortnightly to the nearest mm with a tape measure, as an estimate of horn growth.

STATISTICAL ANALYSIS

Experiment 3 involved two distinct time periods: Period 1 (weeks 1 to 15; 22 December until 31 March) when Treatment groups B, C and D all received bromocriptine administration and treatment Period 2 (weeks 16 - 23, 1 April until 31 May) when Treatments B and C received bromocriptine treatment, C concurrently received prolactin and Treatment D received 'Sulpiride'.

The daily VFI was averaged over each week for each animal and expressed per kg of metabolic liveweight. The logarithm of these values was taken for each animal and differences between treatments assessed in Periods 1 and 2 by analysis of variance (Genstat 2:5.1 Rothamstead Agricultural Station).

Mean live weights for each treatment at the beginning of the experiment and at the end of Periods 1 and 2 were also compared by analysis of variance.

Values for the logarithm of plasma prolactin concentration + 1 were calculated. A regression was fitted of these values against time for each animal in treatment Periods 1 and 2 separately. As in Experiment 2 the y-axis (logarithm of prolactin concentration +1) was repositioned to the midpoint of the x-axis, so that the intercept value was equivalent to the mean value of prolactin concentration within that time period for that individual. Values of mean prolactin concentration and the slope of the fitted line were obtained in this manner for each animal in each treatment period. Differences between treatments in the values of mean concentration and slope were compared independently, examining the differences both between groups within each

treatment period and between groups and within groups across Periods 1 and 2. For both concentration and slope, graphs of residuals vs fitted values and residuals vs normal quantiles were drawn. These demonstrated equal scatter and a straight line relationship respectively, indicating that analysis of variance was appropriate for these data.

The proportion of cashmere in each of the five patches clipped from each animal was calculated from the weight of cashmere divided by the total weight of cashmere plus hair. The weight of the sample was not used as this included debris which was removed during the separation process. The mean proportions of cashmere for each treatment were compared by analysis of variance.

Cashmere content scores and scores on the degree of moulting were firstly expressed as the proportion of animals in a particular category. The 'G' Statistic (Sokal and Rohlf, 1989) was used to test the null hypothesis that moulting was independent of treatment. Williams correction factor for continuity was also used, which reduces error in the 'G' test when a small sample size is used (Sokal and Rohlf, 1989).

The data was also expressed graphically to illustrate the change from winter to summer coat in each treatment group.

RESULTS

Voluntary feed intakes: The mean daily VFI's for each animal in each period expressed as g/kgW*0.75/day, are presented in Table 1. The logarithmic values of the mean daily VFI are described in Figure 1. In Period 1 the VFI by animals in Treatment C was significantly ($p<0.05$) lower and in Treatment D significantly ($p<0.05$) higher than the other treatments. From Figure 1 it can be seen that the VFI's in Treatment C were lower throughout the period whilst the VFI's in Treatment D were similar to that of the Treatments A and B in the first part of Period 1, but were higher subsequently. In Period 2, the VFI's of goats on Treatment B were significantly ($P<0.001$) lower than Treatments A, C and D. There were no significant differences between the other treatments in Period 2 with VFI's increasing steadily over the period.

Live weights: The mean live weights at the start and end of each period are presented in Table 2. There were no significant differences in live weight between the treatments at any time.

Plasma prolactin concentrations: Mean weekly plasma prolactin concentrations are shown in Figure 2 for each treatment. As discussed above a regression was fitted to the log (mean plasma prolactin concentration +1) for each individual in treatment periods 1 and 2.

In Period 1, animals in Treatment A showed a gradual increase in plasma prolactin concentration with time, whereas those in Treatments B, C and D exhibited no increase. Treatment A animals had significantly ($p<0.001$) higher mean plasma prolactin concentrations than those in Treatments B, C and D. Table 3.

In Period 2, Treatment A continued to increase with time. Animals in Treatments C and D demonstrated sharp increases in plasma prolactin concentration in Period 2, whereas Treatment B had a very slight increase at

Table 1. Mean daily VFI (g/kg w0.75/day) for each treatment group in each week**

Treatment	WEEK 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
A: Control (n=10)	46.6	49.0	51.0	56.2	56.5	59.3	58.6	60.5	53.5	57.7	49.9	55.5	62.3	49.9	57.0	70.4	74.9	76.2	79.0	80.3	82.0	83.4	83.9
B: Bromocriptine (n=10)	40.0	53.3	49.0	55.7	52.0	60.3	57.2	63.8	55.1	62.0	55.1	59.1	55.8	36.1	43.5	50.3	53.7	61.5	66.4	68.2	70.2	73.1	72.9
C: Bromocriptine plus Prolactin (n=10)	27.7	39.6	32.4	42.9	45.3	53.7	48.8	51.0	47.0	56.1	51.1	56.9	50.0	39.9	52.5	67.8	74.3	75.8	78.6	79.3	82.3	83.7	84.2
D: Bromocriptine plus Sulpiride (n=10)	38.7	49.5	50.3	60.7	59.1	68.2	66.3	69.7	58.1	65.1	61.4	73.1	75.8	59.0	53.8	59.6	69.6	82.8	86.9	87.4	88.6	88.7	89.2
mean s.e.d.	max-max		9.52																				

Figure 1. Mean daily VFIs (g/kg lwt0.75) for each group over both treatment periods.**

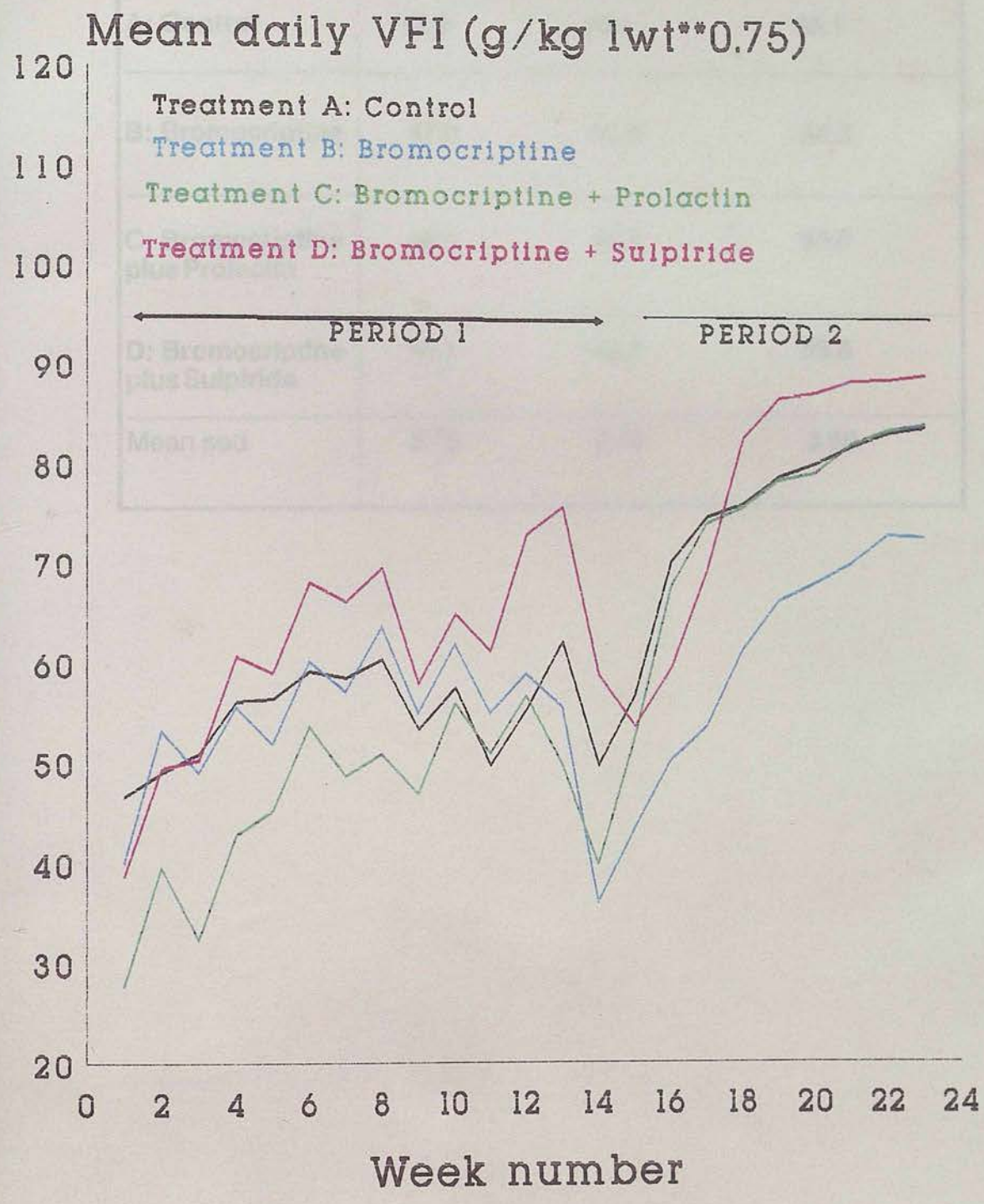


Table 2. Mean live weight (kg) of each treatment group

Treatment	Start of Expt 22.12.89	End of period 1 2.4.90	End of period 2 31.5.90
A: Control	48.6	50.4	55.1
B: Bromocriptine	47.0	50.6	54.3
C: Bromocriptine plus Prolactin	46.0	48.7	53.0
D: Bromocriptine plus Sulpiride	46.1	49.3	53.8
Mean sed	3.76	3.78	3.69

Figure 2. Mean plasma prolactin concentrations of each group over both treatment periods.

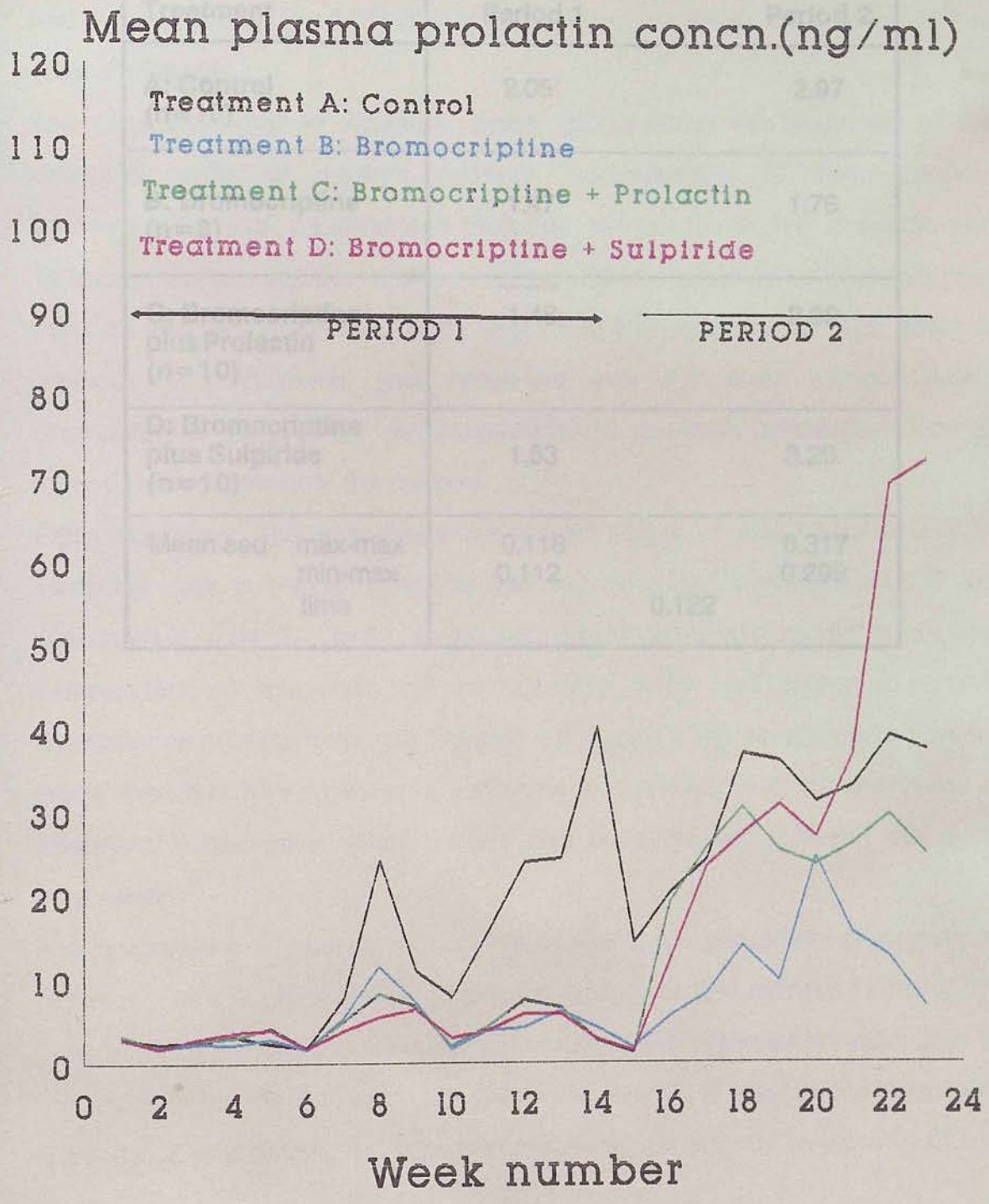


Table 3. Mean plasma prolactin concentration (ng/ml) for each treatment from regression of logn (plasma prolactin concentration + 1) on time.

Treatment		Period 1		Period 2
A: Control (n=10)		2.05		2.97
B: Bromocriptine (n=8)		1.47		1.76
C: Bromocriptine plus Prolactin (n=10)		1.48		2.99
D: Bromocriptine plus Sulpiride (n=10)		1.53		3.20
Mean sed	max-max	0.118		0.317
	min-max	0.112		0.299
	time		0.122	

the end of Period 2. The mean plasma prolactin concentrations of Treatment B were significantly ($p<0.01$) lower than the other treatments (Table 3).

From the results in Table 4, it is evident that there were no significant differences in slope between any of the Treatments in Period 1. In Period 2, Treatment D had a significantly ($p<0.05$) higher regression coefficient than those of the other treatments.

The data illustrated in Figure 3, clearly demonstrate the response of the circadian cycle of plasma prolactin concentration to bromocriptine, bromocriptine plus prolactin and Sulpiride treatment In the prolactin and 'Sulpiride' treated animals, prolactin concentration increased immediately post injection and for up to four hours after, and then decreased. The effect of prolactin and 'Sulpiride' was consistent over the three sample dates. Bromocriptine convincingly suppressed plasma prolactin concentration on all three dates compared to the controls.

Fibre shedding: The mean cashmere content scores of each treatment on the sampling date in Period 1 and at the beginning and end of Period 2 are presented in Table 5. On 13 March and 10 April, cashmere content score was independent of treatment, but on 28 May there was highly ($p<0.001$) dependence on treatment, with Treatment B having a higher cashmere content score than the other treatments, indicating that no shedding of the cashmere in treatment B had taken place. There was no difference between the other treatments.

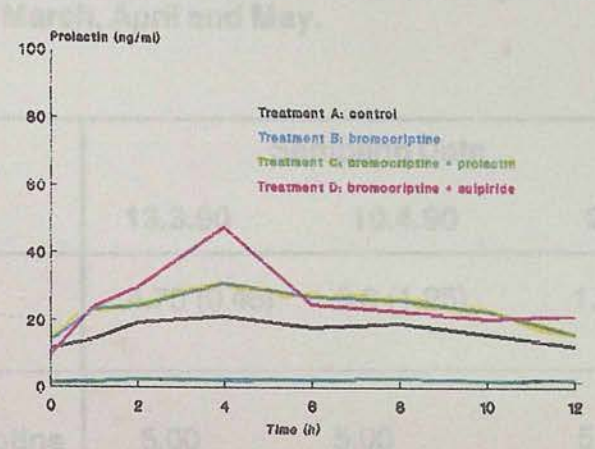
The proportions of patches with scores of 4 or 5 are presented graphically in Figure 4. As the proportion of patches scoring 4 or 5 is representative of the proportion of animals that have not yet moulted, this provides an illustration of the timing of the moult in each group. From Figure 4 it is clear that in Treatment B the moult was delayed until the end of the experiment. In Treatments C and

Table 4. Mean slope of fitted regressions of logn plasma prolactin concentration + 1 on time in Periods 1 and 2.

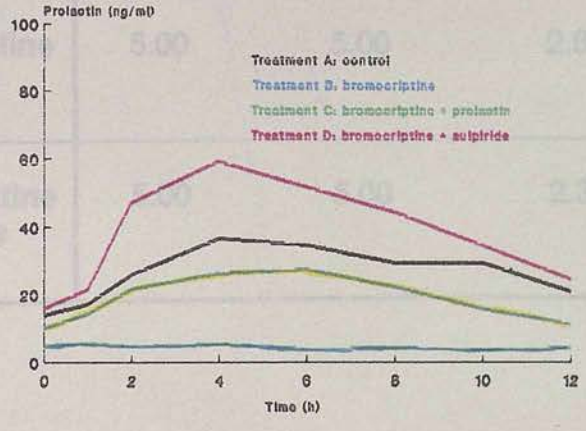
Treatment	Period 1	Period 2
A: Control (n=10)	0.15	0.38
B: Bromocriptine (n=8)	0.00	0.17
C: Bromocriptine plus Prolactin (n=10)	-0.27	0.30
D: Bromocriptine plus Sulpiride (n=10)	-0.15	0.81
Mean sed max-max min-min time	0.270 0.282 0.066	0.180 0.191

Figure 3. Plasma prolactin concentrations of animals in each group sampled for up to 12 hours post treatment injection.

a. 04.04.90



b. 01.05.90



c. 29.05.91

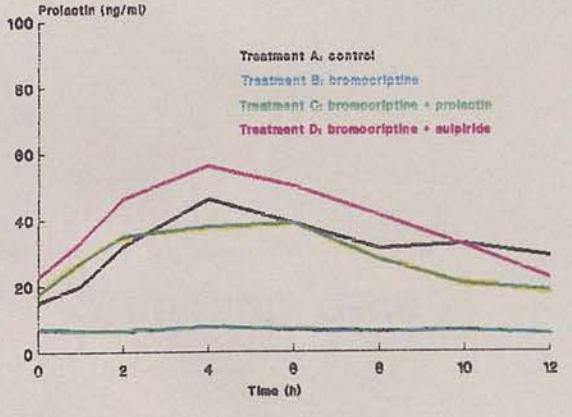
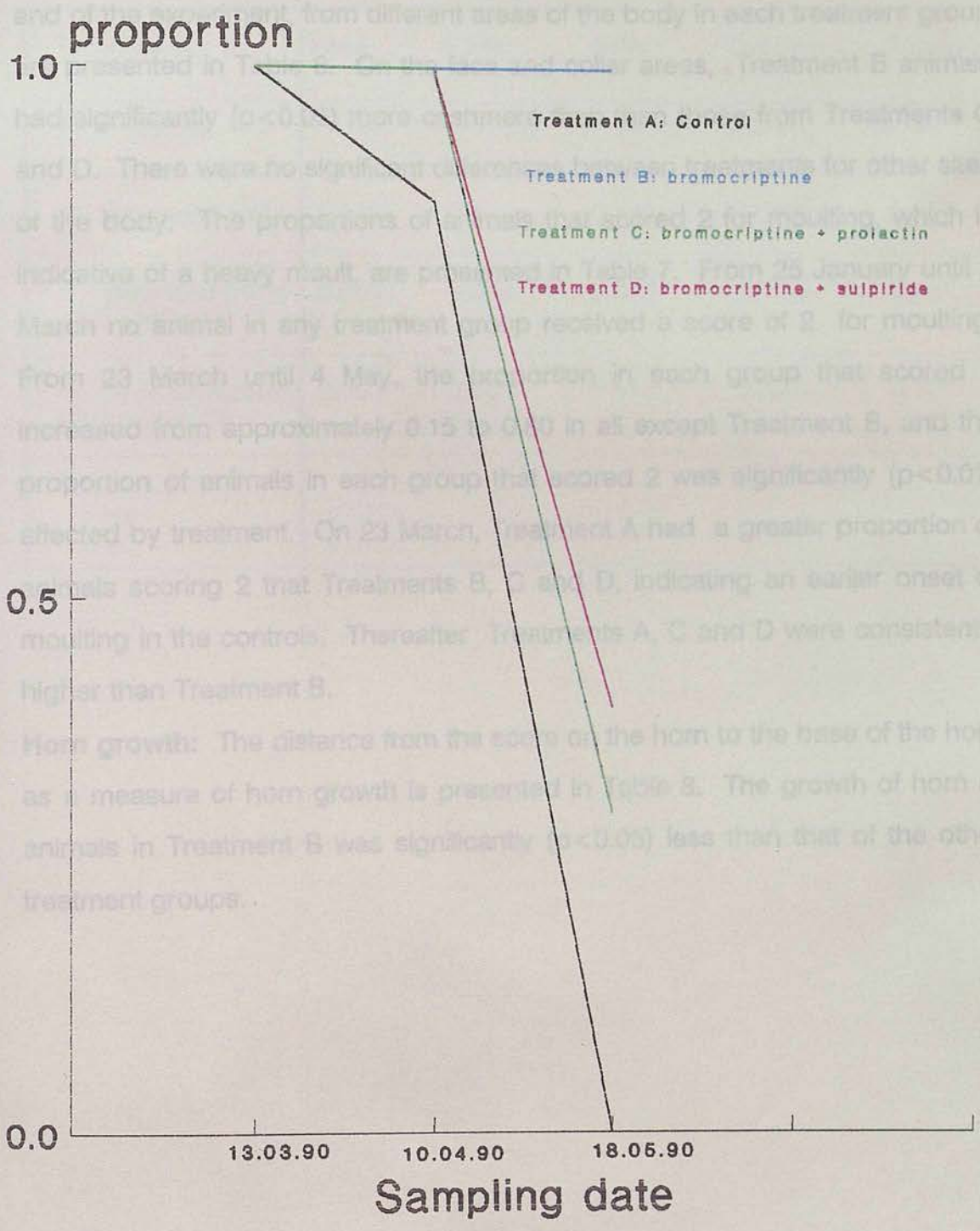


Table 5. Mean cashmere content score (se in parenthesis) of each group on March, April and May.

Treatment	Sampling Date		
	13.3.90	10.4.90	28.5.90
A: Control	4.75 (0.46)	3.8 (1.25)	1.50 (0.93)
B: Bromocriptine	5.00	5.00	5.00
C: Bromocriptine plus Prolactin	5.00	5.00	2.60 (1.71)
D: Bromocriptine plus Sulpiride	5.00	5.00	2.30(1.89)

Figure 4. Proportion of clipped patches in each group that scored 4 or 5.



D, the moult was delayed compared to the controls until the end of Period 1, the time of cessation of prolactin suppression in Treatments C and D.

The proportion by weight of cashmere in patches clipped on 25 May, i.e. at the end of the experiment, from different areas of the body in each treatment group are presented in Table 6. On the face and collar areas, Treatment B animals had significantly ($p<0.05$) more cashmere than than those from Treatments C and D. There were no significant differences between treatments for other sites of the body. The proportions of animals that scored 2 for moulting, which is indicative of a heavy moult, are presented in Table 7. From 25 January until 9 March no animal in any treatment group received a score of 2 for moulting. From 23 March until 4 May, the proportion in each group that scored 2 increased from approximately 0.15 to 0.80 in all except Treatment B, and the proportion of animals in each group that scored 2 was significantly ($p<0.01$) affected by treatment. On 23 March, Treatment A had a greater proportion of animals scoring 2 that Treatments B, C and D, indicating an earlier onset of moulting in the controls. Thereafter Treatments A, C and D were consistently higher than Treatment B.

Horn growth: The distance from the score on the horn to the base of the horn as a measure of horn growth is presented in Table 8. The growth of horn of animals in Treatment B was significantly ($p<0.05$) less than that of the other treatment groups.

Table 6. Proportion of cashmere in fibre samples clipped from areas of the body at the end of Period 2.

Treatment	Face	Collar	Belly	Topline	Rump
A: Control	0.079	0.000	0.120	0.239	0.155
B: Bromocriptine	0.161	0.140	0.147	0.204	0.212
C: Bromocriptine plus prolactin	0.066	0.069	0.082	0.182	0.241
D: Bromocriptine plus sulpiride	0.003	0.034	0.015	0.328	0.160
mean s.e.d. treat area	 0.0361 0.0365				

Table 7. Proportion of animals that scored 2 for moulting i.e. moulting heavily.

Treatment	ASSESSMENT DATE				
	23 March	6 April	20 April	4 May	19 May
A: Control	0.25	0.38	0.38	0.75	0.25
B: Bromocriptine	0.13	0.13	0.25	0.13	0.25
C: Bromocriptine plus prolactin	0.10	0.50	0.50	0.90	0.90
D: Bromocriptine plus sulpiride	0.10	0.10	0.60	0.80	0.80

Table 8. Horn length (mm) measured after marking at 50mm on 23 March.

Treatment	23 March	4 April	20 April	4 May	25 May
A: Control (n=8)	50.00	52.29	57.71	60.43	66.29
B: Bromocriptine (n=9)	50.00	50.12	52.87	57.00	60.75
C: Bromocriptine plus prolactin (n=8)	50.00	50.44	56.78	59.11	65.44
D: Bromocriptine plus sulpiride (n=9)	50.00	50.33	55.11	58.56	65.33
mean s.e.d.	min-min 0.677 min-max 0.658 max-max 0.638				

DISCUSSION

As in Experiment 3, the aim of the design of this experiment was to manipulate plasma prolactin concentrations as a means of investigating its relationship to changes in the hair growth and moulting cycle. Figure 2 clearly demonstrates that differences in plasma prolactin concentrations were obtained. In Period 1, when Treatments B, C and D all received bromocriptine, animals in the control group had a significantly higher mean plasma prolactin concentration ($p<0.001$) than those on the three treatments; baseline levels were obtained with bromocriptine treatment. The control values were similar to those obtained in previous experiments. No significant differences were observed in the change of plasma prolactin concentration with time indicating no differences in the rate of increase or decrease over Period 1.

In Period 2, attempts were made to elevate plasma prolactin concentrations by the use of 'Sulpiride' or by direct exogenous ovine-prolactin administration in Treatments D and C respectively. The bromocriptine-treated group had significantly lower mean plasma prolactin concentrations than the control group ($p<0.001$). Although the plasma prolactin concentrations were elevated by 'Sulpiride' and by exogenous ovine-prolactin, compared to the levels achieved by Treatment B they only achieved the level of the controls, which were experiencing photoperiodically regulated prolactin concentrations. The aim was to elevate plasma prolactin concentrations in Treatments C and D from concentrations that were significantly lower than the controls in Period 1 to significantly higher than the controls in Period 2, and this was not achieved. Treatment D, was however effective in producing a more rapid increase in plasma prolactin concentration.

Although bromocriptine treatment convincingly suppressed plasma prolactin concentrations when compared to the other treatments, a slight increase in

plasma prolactin concentration was also observed in the bromocriptine treated group towards the end of treatment Period 2. This is shown in Figure 2. An increase in plasma prolactin concentration after prolonged bromocriptine treatment was also observed in Experiment 3, and as already noted, this response to prolonged prolactin suppression via a dopamine agonist was also reported by Curlewis *et al* in both red deer (1988) and Scottish Blackface sheep (1991). The time of the rise in prolactin observed in Treatment B animals and in Treatment group D of Experiment 3, are very similar. This supports the argument that decreased sensitivity to bromocriptine is related to the seasonal decrease in dopamine (Curlewis *et al*, 1991).

Voluntary feed intake: The VFIs of the control group over Periods 1 and 2 demonstrated a steady increase over the experiment, suggesting a seasonal increase in appetite as has been previously found in some breeds of sheep (Iason and Mantecon, 1990, Mantecon *et al,* 1989) and deer (Louden *et al*, 1989). However this is not consistent with the results found in Experiments 1 and 3. These differences cannot be satisfactorily explained from these studies, although it should be noted that the goats used in this experiment were either castrates of the feral X Icelandic or feral x Tasmanian breeds, whereas in Experiments 1 and 3, the animals were female domestic (i.e. dairy type) x ferals, which may display less seasonal characteristics in the same way as the Suffolk and Blackface sheep breeds differ (Iason and Mantecon, 1990).

In Period 1, when animals in Treatments B, C and D were all receiving the same amount of bromocriptine treatment, significant differences in VFI were observed. There was no relationship between mean plasma prolactin concentration and VFI for these animals and no explanation can be offered for the differences in VFI. In Period 2, Treatment B animals, which had a

significantly ($P<0.001$) lower plasma prolactin concentration also had a significantly lower VFI ($p<0.01$) than than those in Treatments A, C and D, indicating that the suppression of plasma prolactin concentration at this time may be associated with a suppression in VFI. As a significant difference in VFI was evident in Period 2 between Treatments B and C, i.e. bromocriptine and bromocriptine plus prolactin administration, the lower VFI in treatment B animals can be attributed to a reduction in plasma prolactin concentration rather than to some other effects of the bromocriptine treatment.

Fibre shedding: Animals in Treatment B had a significantly higher cashmere content score on the 28 May at the end of the experiment, than those in Treatments A, C and D. This indicates a delay in the loss of cashmere from the coat of bromocriptine-treated goats; this was associated with a significantly ($p<0.001$) lower plasma prolactin concentration in Period 2. Figure 3, which graphically presents the moulting of the cashmere in each group, demonstrates this delay in cashmere loss in Treatment B animals and also a delay until the end of Period 1, the period of plasma prolactin suppression, in Treatments C and D. The sharper decrease in cashmere content score observed in Treatments C and D demonstrates a more rapid loss of cashmere associated with the increase in plasma prolactin concentration from baseline levels to that of the controls. The proportion of animals in each treatment group that were moulting heavily presents a similar picture to the cashmere content score, in the timing of the spring moult. The onset of moulting from the hair follicles occurs slightly before any noticable loss of cashmere from the coat. This order of events is consistent with the significant differences in the proportion of animals moulting by 23 March, compared to no animals moulting on 10 April for the cashmere content scores. On 23 March the control group had a higher

proportion of animals moulting than did Treatments B, C and D. At this time, B, C and D were all receiving bromocriptine treatment. From 6 April, Treatments C and D had a greater proportion moulting than did A and B. This rapid change in the proportion of animals moulting is associated with the sharp increase in plasma prolactin concentrations in Treatments C and D; the smaller proportion moulting in Treatment B, compared to Treatments C and D, is associated with the suppresion in plama prolactin concentrations. The delay in moulting in Treatments C and D until the rise in plasma prolactin concentration, conclusively demonstrates that an increase in plasma prolactin concentrations is necessary for the initiation of fibre moulting and hence the breaking of the junctional complexes between the hair fibres and the follicle. The sudden change associated with this increase and the resulting condensed moult within each individual in Treatments C and D also suggests greater synchronisation of the moult over the body, compared to the controls.

The proportion of cashmere in $2cm^2$ patches clipped from five areas of the body on 24 May demonstrated a significantly higher proportion, by weight, of cashmere on the face and collar in Treatment B compared to the other treatment groups. It is unclear why differences were not observed for the belly, dorsal and rump regions of the body. This could be due to either small amounts of cashmere present on the belly and the presence of matted cashmere held within the long guard hairs of the top line dorsal hairs and the rump, or due to differential shedding over the body. These results do demonstrate that the collar and face are areas of the body that are useful in assessing the progress of moulting.

Horn growth: There was a significantly shorter length of horn growth from 23 March until 23 May in Treatment B animals compared to those in Treatments A,

C and D ($p<0.05$). If we consider that horn is composed of keratin as is hair then it is not unlikely that the growth of hair is linked to the growth of horn and possibly hooves. An alternative explanation is that the lower intakes observed in Treatment group B were the cause, via a reduction in nutrient supply, rather than a seasonal phenomenon, *per se*.

It can be concluded from this experiment that the spring moult in cashmere goats is regulated by the increase in photoperiodically controlled plasma prolactin concentration. The moult can be delayed by bromocriptine and then induced, in a group of animals. The degree of asynchrony within that group appears to be reduced however other factors other than prolactin may be involved at the level of the individual follicle. It is proposed that prolactin is ultimately involved in in the control of the hair follicle cycle but further research is required to understand the control at the follicular level.

Chapter Six: General Discussion

GENERAL DISCUSSION.

The development of experimental techniques designed to monitor the progress of the spring moult.

To investigate thoroughly the response of the hair follicle cycle and the associated moult to endocrine changes, quantitative methods of assessing the onset and progress of the moult required to be developed. These methods had to be:

a. Practical: Because of the high frequency of sampling required to monitor changes in pelage, measurements had to be as simple as possible, rapid and informative.

b. Repeatable: The same measurement had to be taken several times in consecutive weeks to monitor the moult accurately. It was therefore essential to design methods that did not preclude further sampling. Techniques which are non-invasive are particularly advantageous as they are infinitely repeatable and involve less stress to the animal than techniques which require restraint. This is paticularly important as it is well established that plasma prolactin concentrations increase as a result of stress.

c. Representative: One vital consideration in selecting a sampling method or site, is that it is representative of the animal's coat. For example, moulting on the legs is almost continuous and therefore not representative of the seasonal moult of the body and the loss of cashmere from the coat.

d. Statistically valid: To test the effect of different hormonal profiles on the timing and duration of the moult, quantitative as well as qualitative measurements had to be developed so that statistical analyses could be made. This was achieved by developing a scoring system, to assess the amount of

cashmere present in the coat.

To develop a complete picture of the physiological event termed the moult, measurements on each component of the moulting process were made. These were the loosening of fibres within hair follicles, the loss of cashmere from the coat, the decrease in density of the coat, the growth of the replacement coat and the progression of the moult over the body.

METHODS EXAMINED.

1. Patch sampling: An area of fibre was measured with precision callipers and clipped to skin level using surgical clippers. Accurate measurement of a precise area can be extremely difficult due to the elasticity of skin. Samples are generally taken from the mid-side area as this is considered to be representative of the fleece as a whole; also several samples can be taken from this region without overlap. Traditionally the sample is hand separated into hair and cashmere and the yield of cashmere estimated on a weight basis. Due to the length of time required to separate a sample, the inaccuracy involved in taking the sample and the error due to the very small weights of cashmere obtained compared to debris in the sample and guard hair contamination in the separated sample, it was decided that this method was not practicable in the context of these experiments. It was noted, however, that there were obvious differences in the amount of cashmere present and in the density of cashmere between samples. To quantify these differences a photographic reference scale was established similar to that of Duncan and Goldman (1984) for changes in pelage in Djungarian hamsters. The five point scale, 5 - (very dense cashmere present) to 1 - (no cashmere present), is presented in Plates II-V!. At the end of an experiment, all the samples were collected together and

presented to the operator in a random manner. Each sample was compared to the reference scale and scored accordingly. This method was found to be efficient and informative and was used in each experiment.

The proportion of fibre by weight was used in Experiment 3 on patch samples ($4cm^2$) taken from different areas on the animal, i.e. the face, the collar, the mid-side, the dorsal top-line, and the rump. A subsample of about 0.1g was taken from each sample, separated with forceps into cashmere and hair, and each component weighed. Significant differences were obtained between groups of animals for the face and the collar areas but not for the other three regions. From observations noted at the time of sampling differences in the amount of cashmere in these areas between groups of animals were apparent; these differences, however, could not be detected by this method. The proportion of cashmere to hair on the body is less in the torso regions than on the extremities due to shorter guard hair in these areas. The very small proportions of cashmere by weight at any time of year in the samples from the mid-side, top-line and rump make it difficult to detect other than very large differences in the proportion of cashmere fibre. In an attempt to quantify the level of moulting a scoring system was developed. Prior to scoring, the hand was rubbed briskly over the coat to remove any broken or loose fibres, an area of hair was then placed between the forefinger and the thumb and the hand brought firmly down the length of the hair. The number of hairs released was noted. The scale ranged from 0 - no hairs released, 1- few hairs released, 2 - copious quantities of hair released. As each animal was individually known there was obviously the possibility of preemptive bias. Therefore, each animal was always scored by the same operator and each score was verified by an independent witness. This system had many advantages in that it was quick, efficient, repeatable, had a low stress factor, and was simple to operate. The main

disadvantage was that the moulting of guard hairs is visibly more obvious and therefore the results represent the presence or abscence of primary rather than secondary follicle moulting. However when these results are used in conjunction with cashmere loss from the coat they are correllated to describe the complete moult adequately.

2. Plucked samples. Doney and Smith (1969) described a technique which was used to give a quantitative estimate of the extent of brush-end formation. This involved plucking a sample of 100-200 hairs, and immediately mounting them on a slide with euparal. The fibre ends were examined under a light microscope and the proportion of brush ends (telogen) and torn fibre ends (anagen) counted. This technique which was developed for sheep, was not found to be suitable for cashmere goats. Examination of the slides was time consuming and the information obtained did not provide any additional information additional to that obtained from the body scoring system. To obtain the plucked samples to estimate the proportion of telogen and anagen fibres, the sample was plucked with greater force than for the scoring system. The telogen fibres would be pulled from the follicle bed breaking the junctional complexes. It was concluded that although this technique would estimate the extent of brush-end formation it would not determine the timing of the moult and the breaking of the junctional complexes between the hair and the follicle bed, and therefore was not providing additional information to that supplied by the scoring system and the histological examination of the follicles.

3. Histology:

The examination of the histology of hair follicles is a technique that has been extensively used in hair biology (Lyne and Short, 1965; Ryder, 1965b; for

reviews). The technique includes, fixation of the sample, dehydration of the sample in increasing concentrations of alcohol, embedding in wax under pressure, sectioning, staining and microscopic examination. From the examination of the skin, the stage of the hair cycle of each follicle can be determined and thus the proportion of active follicles estimated. Although the preparation of the slides is a long process, in Experiments 1 and 3, it was considered necessary to conduct histological examinations to support the results concluded from the other techniques. As the timing of the moult obtained from histological results, closely corresponded to that obtained from the cashmere content scores and the visual assessments, it was concluded that cashmere content scoring and visually assessing the animals were sufficiently accurate techniques to be used without histological measurements in Experiment 4.

4. Dye banding:

Areas of fibre on the mid-side position were dyed using commercial hair dye (Borne Blonde, Clairol Jet Black). The regrowth of the original colour was measured using small staple samples from the dyed area at four week intervals. The technique was found useful to measure fibre growth, but the asynchrony within a small area in the replacement of fibres resulted in staple lengths that were impossible to measure. Photographs of samples from two animals are presented in Plates XV and XVI. Dye banding and complete dying of the animals coat has been used in rats to demonstrate the waves of hair replacement over the complete animal. It was concluded that dying areas of goat fibre could be a useful tool when assessing rates of fibre growth but after initial studies it was decided to concentrate on the moulting rather than the growth of cashmere.

APPLICATIONS OF THIS RESEARCH TO THE CASHMERE INDUSTRY.

The consistent effectiveness of bromocriptine in suppressing plasma prolactin concentration and delaying the moult clearly indicates that this could be a the basis of a practicable means by which to delay moulting. It must be noted, however, that the response to bromocriptine is not indefinite and towards the end of the bromocriptine treatment periods (late May), plasma prolactin concentrations were beginning to increase and some animals were in the first stages of moulting. It would therefore be impossible by this method, and at these dose rates, to delay moulting until June or July, but moulting could be delayed until mid-May when, at least in some parts of the UK, housing would not be required. The method and potential cost of administration of bromocriptine (deep intramuscular injections) may be unacceptable to some goatkeepers. Therefore experiments investigating different methods of administration e.g. oral dosing or slow release implants, and different dose rates for each method are required before 'on farm' methods can be established.

Methods of synchronising the moult by periods of prolactin suppression followed by a period of prolactin elevation were investigated in Experiment 4. 'Sulpiride', a dopamine antagonist, was used as an alternative to prolactin which is not readily available. The results indicated that there was some degree of synchronisation following prolactin suppression in the bromocriptine treated groups. However, in the period of prolactin elevation plasma prolactin levels were not increased above the controls. As a result the hypothesis was not adequately tested and definite conclusions on the ability of this technique to synchronise moulting cannot be drawn although the rapid onset of moulting in each individual in Treatments C and D did suggest an increased synchrony over the body and a more condensed moulting period. What these results do

emphasise is that dose levels have to be tested on different sexes, sizes and breed types of goats. The goats in Experiment 3, where the elevation of prolactin concentration was above the controls, were female rather than the castrates used in Experiment 4 and were dairy type x feral rather than the Icelandic/Tasmanian x feral genotypes used in Experiment 4.

The effects of bromocriptine treatment in the pregnant or lactating female require to be investigated. Forsyth *et al* (1985) have studied the effects of bromocriptine treatment on pregnant and lactating dairy goats. No adverse side effects were discovered, although there was a slight reduction in milk production and delay in colostrum let down. Although this was not adverse to the diary goat kid, it is possible that in the fibre producing goat which produces less milk and may be kidding in less intensive surroundings, that the reduction in milk and delay in colostrum could be critical.

THE MECHANISMS CONTROLLING THE SPRING MOULT IN THE CASHMERE GOAT.

The Role of Melatonin.

It has been well established that seasonal cycles in reproduction, appetite and pelage changes are entrained to an annual cycle by the daily pattern of melatonin secretion from the pineal gland. In the cashmere goat, the spring moult and growth of the summer coat, coincides with increasing daylength and a reduction in the duration of melatonin secretion.

In Experiment 1, based on the results of Martinet *et al* (1981) and Smith *et al* (1987a), it was hypothesised that slow-release implants of melatonin, given from mid-December, would maintain high levels of circulating melatonin and prevent the animal becoming sensitive to increasing daylength. As a result they

would not exhibit the photoperiodically governed increase in plasma prolactin concentrations, or the moult to a summer coat. The hypothesis was not supported by the results. The adult animals that received melatonin treatment demonstrated an advance in the seasonal rise in prolactin, normally associated with increasing daylength, and an advance in the timing of the spring moult. In the juvenile goats no differences were observed between the melatonin treated animals and the controls. It appears that in the adult goats, a continuous release of melatonin was not read by the brain as a prolonged period of short daylength, and they became refractory to the melatonin, in advance of the controls.

Recent work in hamsters by Maywood *et al,* (1992) has demonstrated that when interpreting the melatonin signal, the brain is also sensitive to the intervening free period between successive melatonin signals, and that the melatonin profile is read relative to this interval. Occlusion of the melatonin-free interval, by infusions of melatonin, rendered the animal insensitive to the melatonin signal and resulted in reproductive changes consistent with a long day response. The response of putative melatonin binding sites to continuous exposure of melatonin was also investigated by Maywood *et al* (1991). It has been proposed that one of the modes of action of melatonin is to down-regulate its own receptors (Reiter, 1983) and it was hypothesised by Maywood *et al* (1991) that differences in the responses of animals to phasic or continuous exposure to melatonin could possibly be a consequence of reduced availability of the melatonin binding sites, resulting from occlusion of the melatonin-free interval. However, *in vitro* localisation of the iodo-melatonin binding sites, from different groups revealed no differences in the density or distribution of binding sites after either prolonged absence or continuous infusion of melatonin. In addition, no differences in putative melatonin

receptors or melatonin receptor-mediated inhibition of cAMP accumulation, distribution and density were found between control and photorefractory hamsters (Weaver *et al,* 1991) or between two populations of mice, one melatonin sensitive and one insensitive (Weaver *et al,* 1990). These results suggest that sensitivity to melatonin is regulated at levels downstream from the response to acute receptor occupation.

From the above findings and the results of Experiment 1, it can be suggested that elevation of plasma melatonin concentrations with slow-release implants is not an effective method of prolonging the short-day hair follicle response. It should be noted however, that melatonin implants given in late winter-early spring, were effective in preventing the photoperiodically regulated increase in prolactin concentration, and the spring moult in mink. It can therefore only be concluded that this method of delaying the moult is not effective in the female-cashmere goat.

As discussed above, no significant differences were observed between the control and melatonin-treated juvenile goats. It is unclear why this difference in response to melatonin should have occurred between the adult and juvenile goats. Further studies investigating the regulation of the first coat change and possible associations with puberty are required in this area.

The role of prolactin

It can be suggested from the results obtained in Experiments 1 and 3 that the effects of melatonin on the hair follicle cycle are mediated through changes in plasma prolactin concentration and that manipulation of plasma prolactin concentration may lead to manipulation of the hair follicle cycle.

It was hypothesised from the results of Experiment 1 that the seasonal rise in

plasma prolactin concentration, which is regulated by changes in melatonin secretion in response to photoperiod, controls the onset of moulting and the reactivation of the hair follicles. This hypothesis was supported strongly by the resutls from Experiments 3 and 4. It was conclusively demonstrated that the suppression of plasma prolactin concentration with bromocriptine prevented the onset of moulting and reactivation of the hair follicles. This effect of bromocriptine was completely overcome by concomittant prolactin administration in the form of subcutaneous injections. Furthermore the group were treated with prolactin when endogenous levels were low and showed an advanced moult compared to the control group. It can be concluded from these results that changes in plasma prolactin concentration are important for reactivating the hair follicles after telogen and hence the moult from winter to summer pelage.

It can not be stated from these results that prolactin directly has an effect on the hair follicle. It is probable that prolactin exerts its effect on the follicle via an intermediary, e.g. a local growth factor. Consistently throughout this study the question of asynchrony between individual hair follilces in an individual and asynchrony between a group of animals has arisen. Asynchrony within an animal is an obvious advantage in that it prevents the animal losing the majority of the coat simultaneously and the obvious thermoregulatory hazards. If the hair follicle was responding directly to changes in plasma prolactin concentration, the asynchrony could be explained by hair follicles having different abilities to respond to the prolactin signal e.g. different numbers or affinities of receptors. To elucidate what is directly controlling the individual follicle it is necessary to identify and characterise binding sites on the follicle (Shulster and Levitzki, 1980) as every hormone, neurotransmitter and drug must exert its biological affect by binding to a specific receptor. Epidermal

growth factor binding sites have been identified in the merino sheep (Wynn *et al,* 1990) and epidermal growth factor has frequently been associated with hair growth in mice (Moore *et al,* 1981,1983) and sheep (Moore *et al,* 1982).

The role of prolactin in regulating the seasonal coat cycle in cashmere goats has been conclusively demonstrated, but the mechanisms through which it exerts its action remain to be identified. Due to the involvement of prolactin in many physiological events, it would be advantageous to control the hair follicle directly to manipulate hair growth and hair loss, to prevent other unwanted responses to treatment.

Plate I A scanned electron-micrograph of the skin surface of a cashmere goat. Both the coarse guard hairs and fine cashmere fibres have coronet-like scales (After Blazej *et al,* 1989).

Plates II-VI A photographic reference scale developed to score patches of fibre on the quantity of cashmere present.

Plate II Score 1: Typical of a summer coat. Sample very sparse and no cashmere present.

Plate III Score 2: Sample quite sparse, only small amounts of cashmere present.

Plate IV Score 3: Sample moderately sparse, small amounts of cashmere present, but less cashmere than hair.

II

III

IV

Plate V Score 4: Sample quite dense, obvious quantities of cashmere present

Plate VI Score 5: Sample very dense, vast amounts of cashmere present. Typical of a winter coat.

V

4.

VI

5.

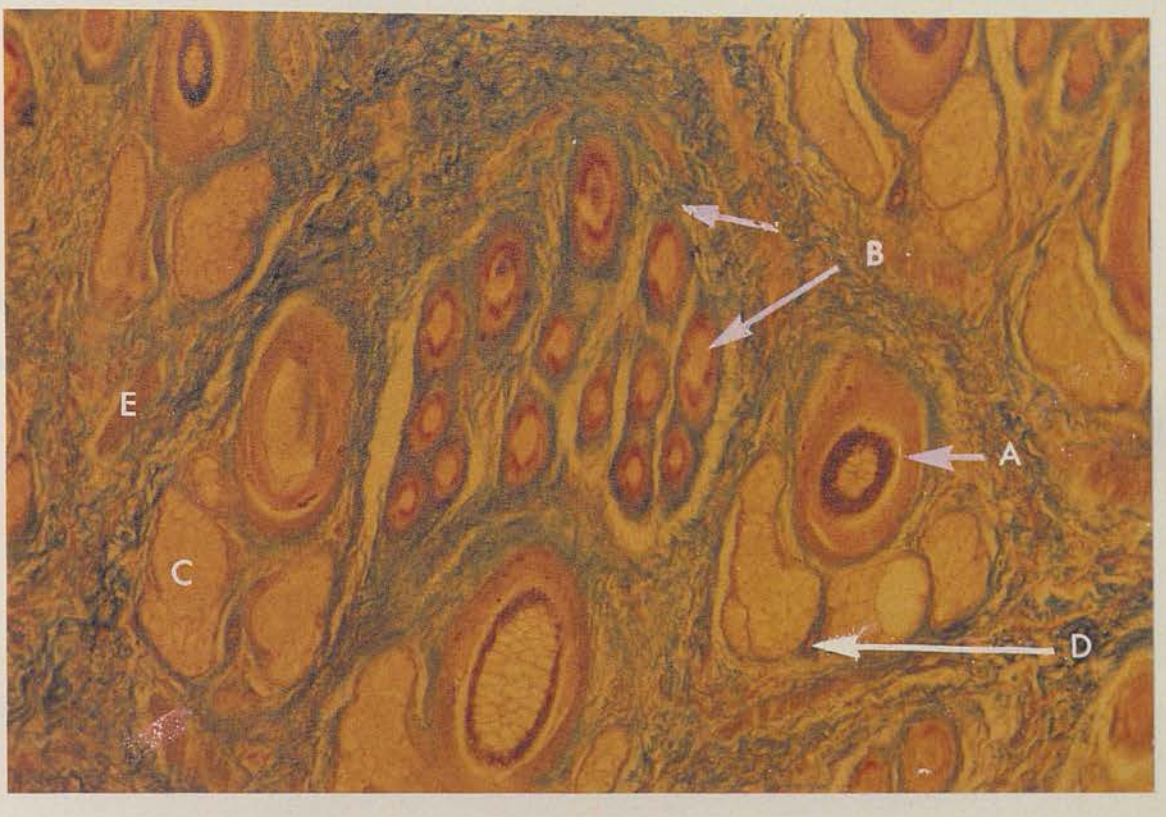
Plates VII-XIV Photographs of transverse sections (8 microns) of goat skin, prepared after the method of Ryder and Stephenson (1968) and examined under a light microscope.

Plate VII A photograph of a hair follicle group, showing a trio of primary hair follicles* (b) positioned between them. Each primary follicle has an associated sebaceous gland (c), sweat duct (d) and erector pili muscle (e). (x40)

* (a) and smaller secondary follicles

Plate VIII The arrangement of hair follicle groups in the skin. (x10)

VII

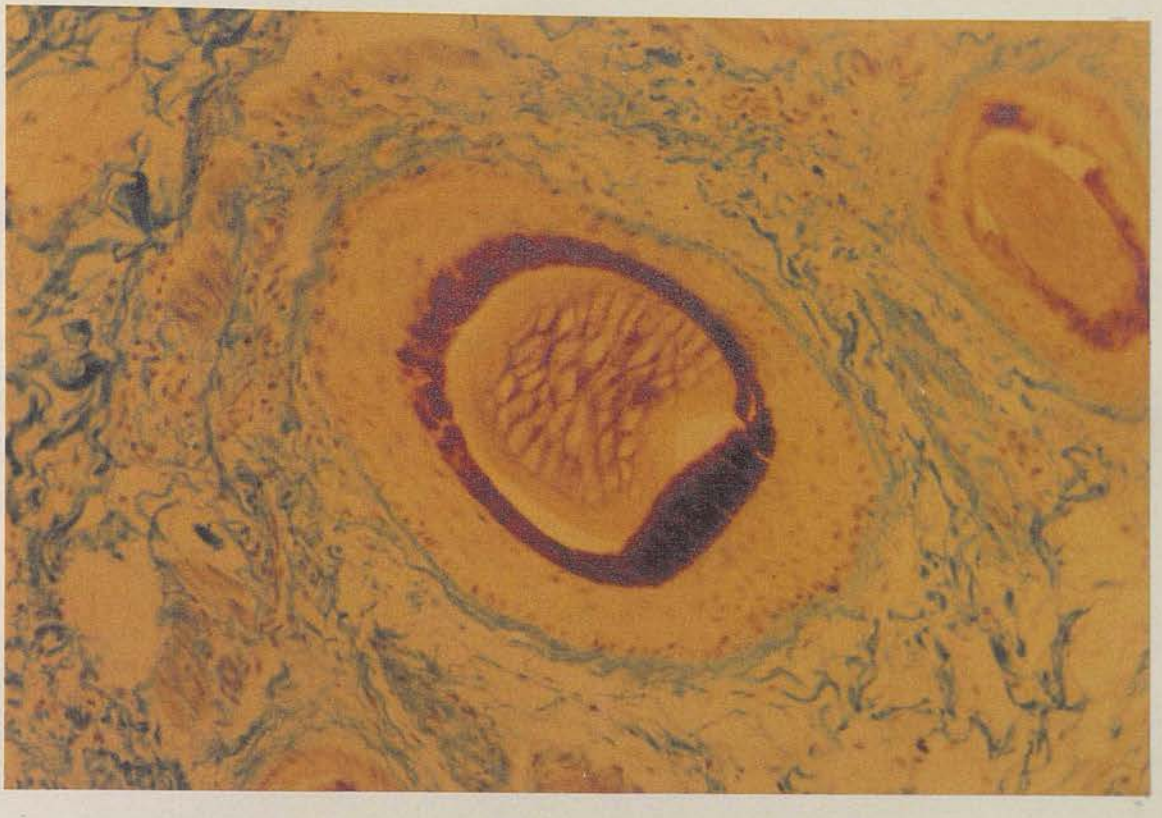


VIII

Plate IX A primary hair follicle in anagen, with a medullated fibre present. The inner root-sheath of the anagen follicle is basophilic and stained red. (x100).

Plate X A hair follicle group in anagen. As in the primary follicles (more obvious in Plate IX) the inner root-sheath stains red. (x40).

IX



X

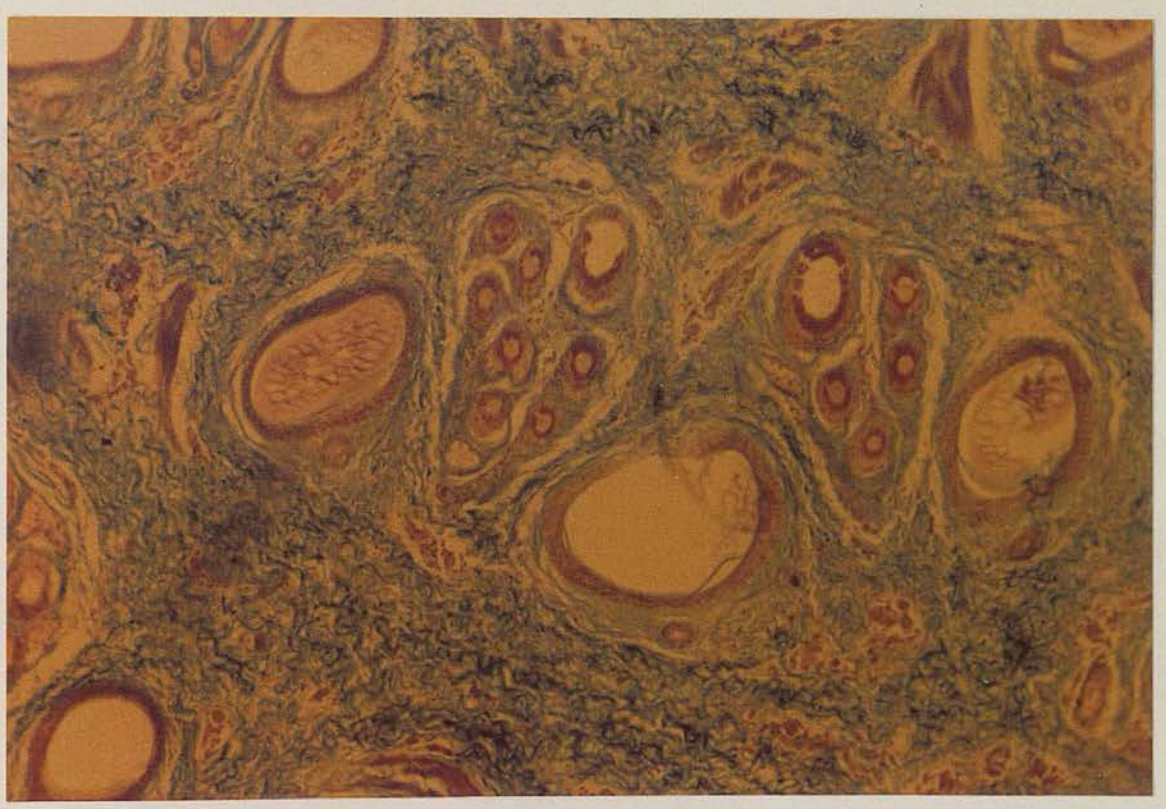
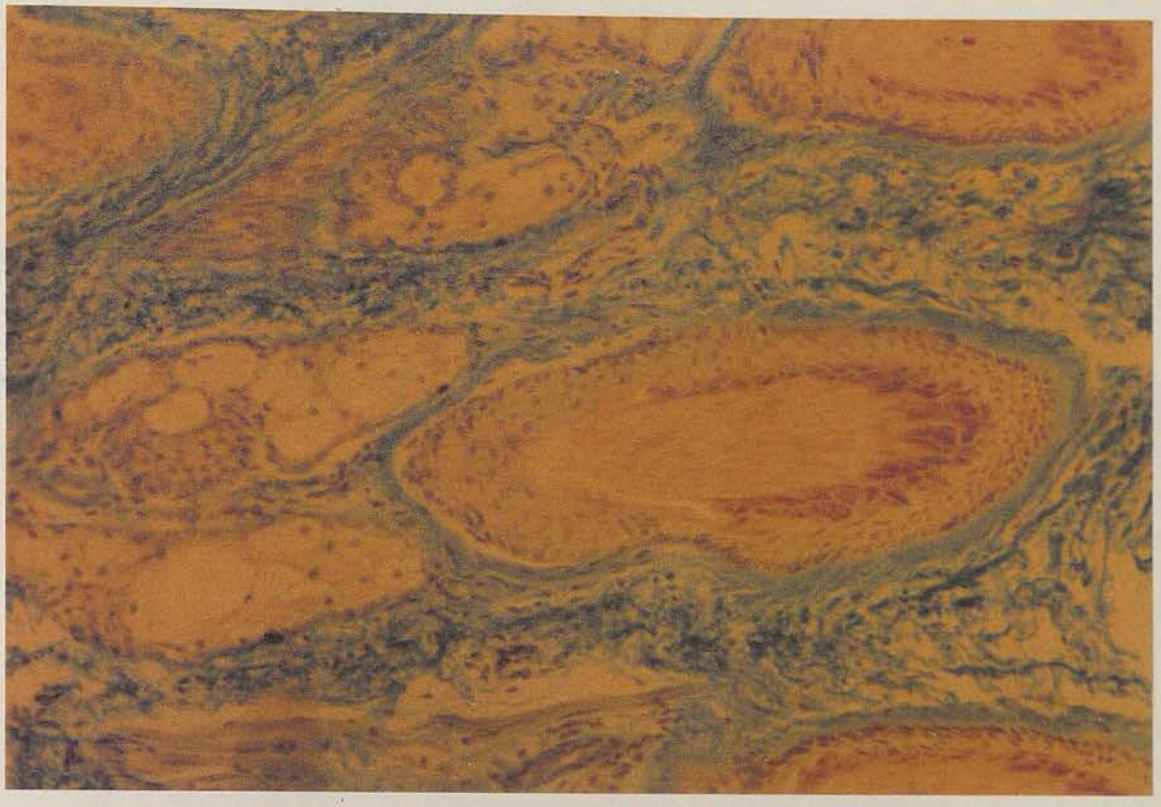


Plate XI A primary hair follicle in telogen, demonstrating the characteristic brush-like shape. The nuclei are stained dark brown and appear to gravitate towards the centre of the follicle. The resting follicle is smaller than the active follicle. (x100).

Plate XII A follicle group in telogen. The dark stained nuclei and brush shape is also evident in the secondary follicles. (x40).

XI



XII

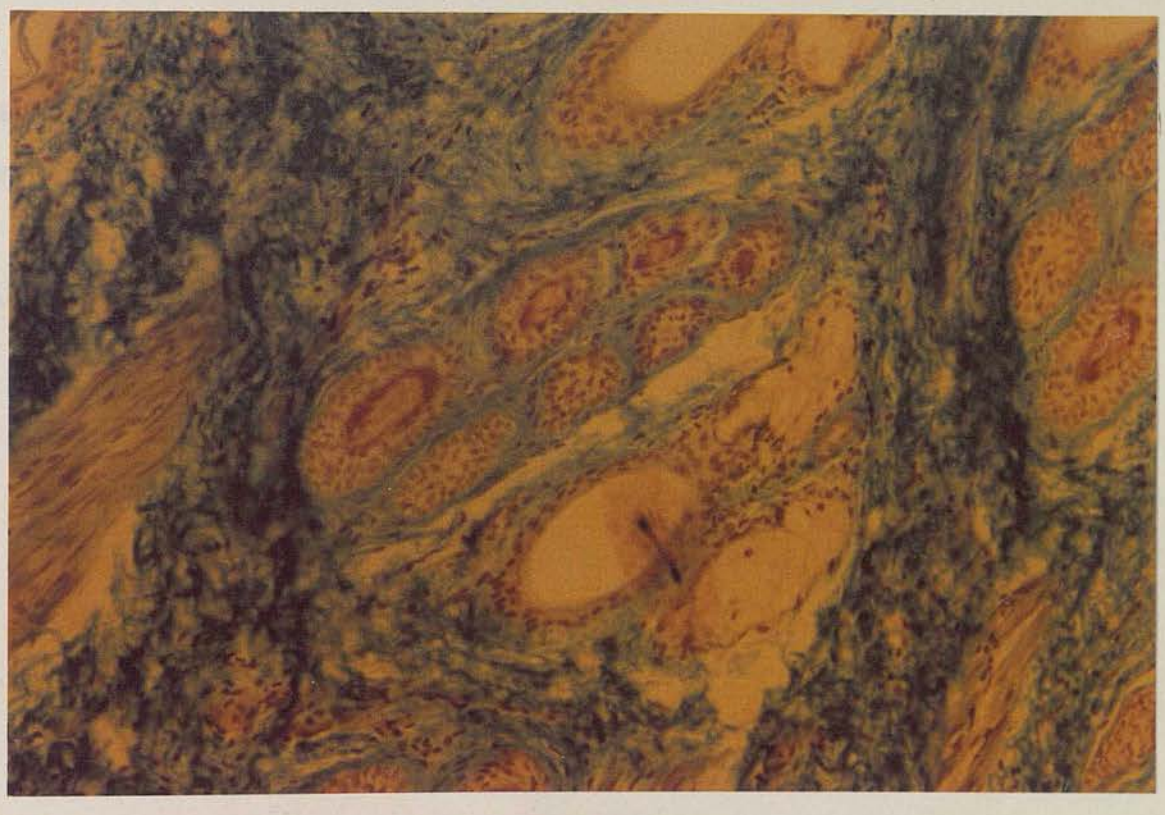
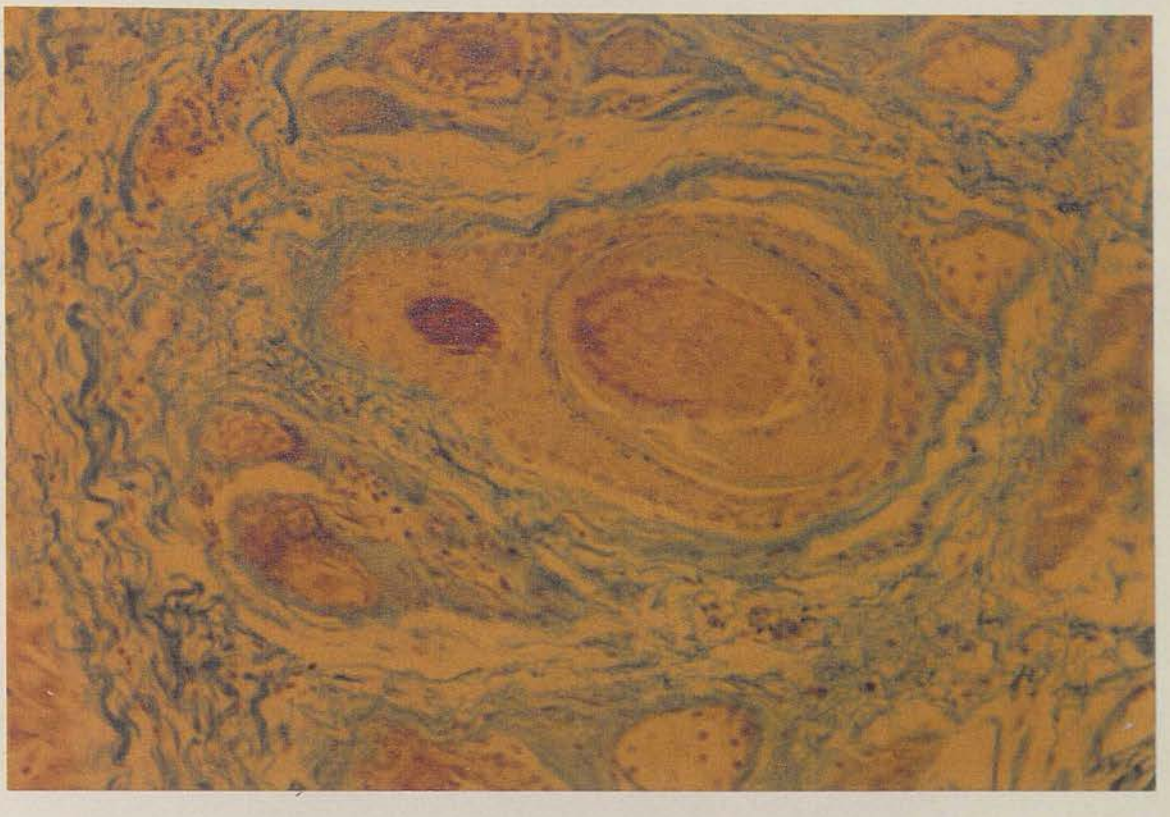


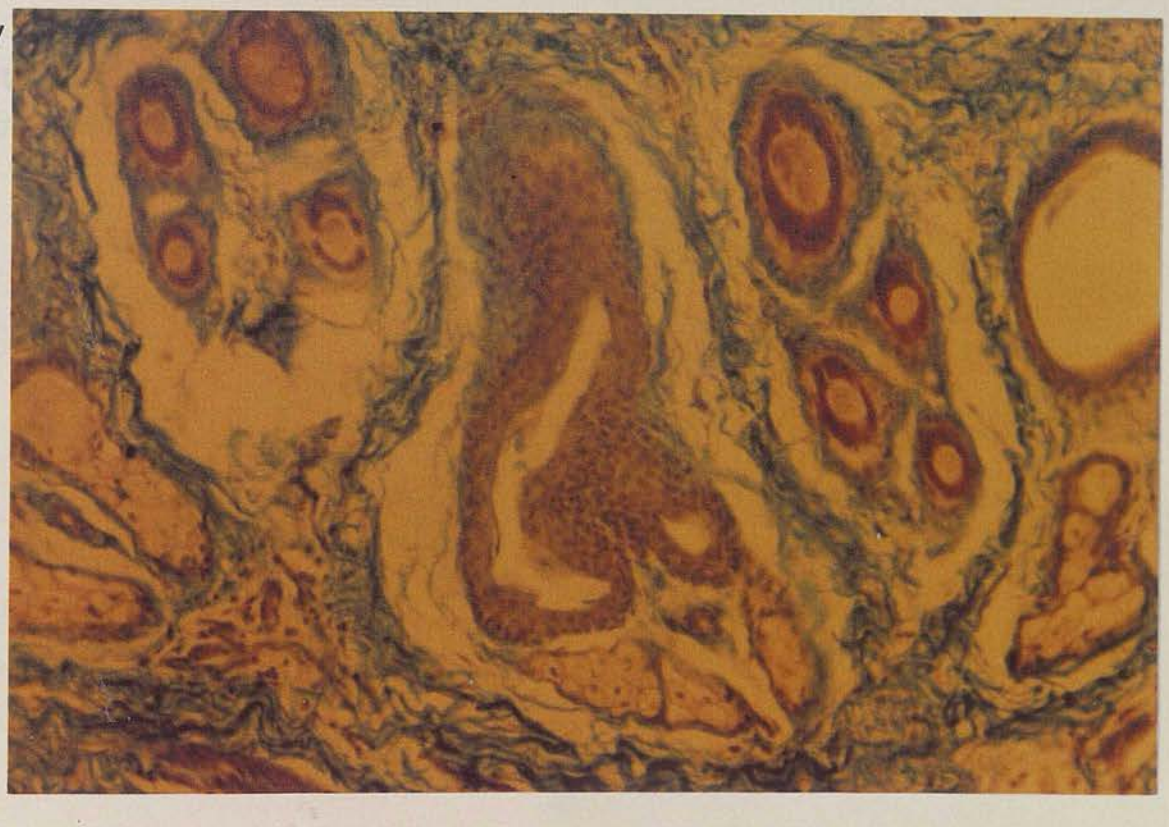
Plate XIII A hair follicle showing a resting mature fibre and a new fibre growing alongside. This was termed a shed regrowth by Ryder (1966). The new fibre is strongly basophilic and stains red.

Plate XIV A collapsed follicle, with nucear staining typical of a resting follicle. It differs from an empty follicle where the fibre has been lost during processing as that retains its shape. This is possibly a follicle in catagen that has shed its mature fibre.

XIII



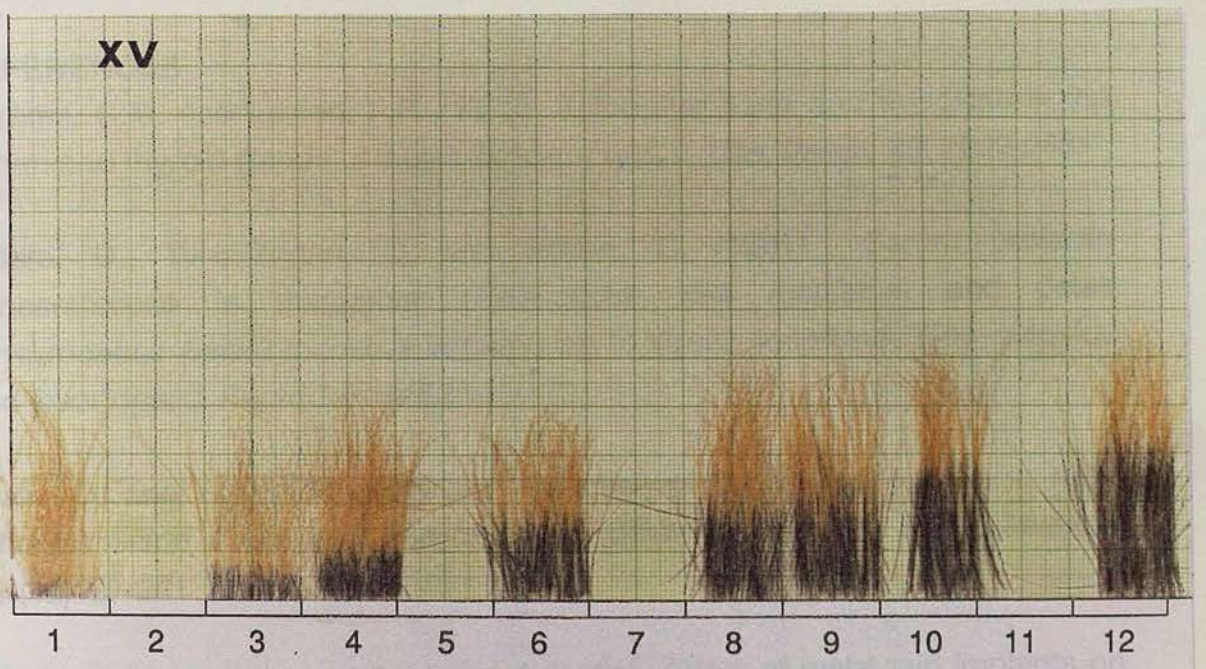
XIV



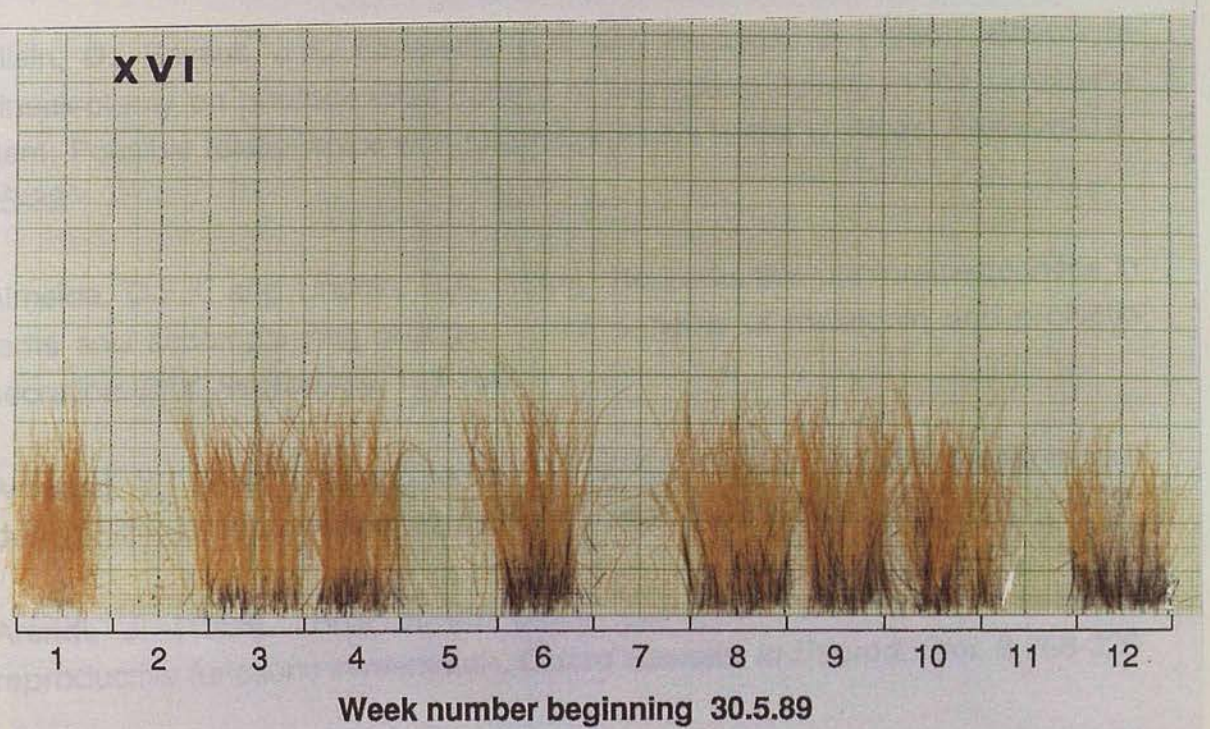
Plates XV and XVI Staple samples of fibre cut from a patch of fibre bleached on 30.5.89.

Plate XV The regrowth in the staple samples are uniform and growth can be measured easily.

Plate XVI Regrowth is asynchronous throughout the staple lengths and regrowth is irregular.



Animal 6134 Experiment 2
Treatment group Bromocriptine plus prolactin



Animal 6143 Experiment 2
Treatment group prolactin

REFERENCES

Abrams, G.M. and Larsen, P.R. (1973) Triiodothyronine and thyroxine in the serum and thyroid glands of Iodine-deficient rats. *J. Clin. Invest.* **52**, 2522-2531

Aidera. D., Tahiri-Zagret, C. and Robyn, C. (1981) Serum prolactin concentrations in mangabey *(Cerrocebus atys lunulatus)* and patas *(Erythrocebus patas)* monkeys in response to stress, ketamine, TRH, sulpiride and levodopa. *J. Reprod. Fert.* **62**,165-172

Allain, D. and Rougeot, J. (1980) Induction of the autumn moult in mink *(Mustela vison Peale and Beavois)* with melatonin. *Reprod. Nutr. Develop.* **20,**(1a)187-201

Allain, D., Martinet, L. and Rougeot, J. (1981) Effect of melatonin implants on changes in the coat, plasma prolactin and testes cycle in the mink (*Mustela vison*) In. *Photoperiodism and Reproduction in Vertebrates.* Eds. R. Ortavant. J. Pelletier and J.P. Ravault.pp263-271. INRA Services Publications. Versailles.

Allain, D., Ravault, J.P., Panaretto, B.A. and Rougeot, J. (1986) Effects of pinealectomy on photoperiodic control of hair follicle activity in the Limousine Ram: Possible relationships with plasma prolactin levels. *J. Pineal Research.* **3**, 25-32

Almeida, O.F.X. and Lincoln, G.A. (1984) Reproductive photorefractoriness in rams and accompanying changes in the patterns of melatonin and prolactin secretion. *Biol. Reprod.* **30,** 143-158

Amenori. Y., Chen, C.L. and Meites, J. (1970) Serum prolactin levels in rats during different reproductive states. *Endocrinology.* **86,** 506-510

Arendt, J. (1986) Role of the pineal gland and melatonin in seasonal reproductive functions in mammals. *Oxford Reviews In Reprod. Biol.* **8,**266-320

Argyris, T.S. and Argyris, B.F. (1970) Hair growth on skin grafts placed on hairless mice. *Anat. Rec.* **168,**457-462

Ben-Jonathan, N. (1980) Catecholamines and pituitary prolactin release. *J. Reprod. Fert.* **58,** 501-512

Bissonette, T.H. (1935) Relation of hair cycles in ferrets to changes in the anterior hypophysis and light cycles. *Anat. Rec.* **63,**157-159

Bittman, E.L. (1978) Hamster refractoriness: The role of insensitivity of pineal target tissues. *Science.* **202**,648-650

Blackburn, P.S. (1965) The hair of cattle, horse, dog and cat. In. *Comparative Physiology and Pathology of the Skin.* Eds. A.J. Rook and G.S. Walton pp201 Blackwell Scientific Publications.

Blask, D.E., Leadem, C.A., Orstead, K.M. and Larsen, B.R. (1986) Prolactin cell activity in female and male Syrian hamsters: an apparent sexually dimorphic response to light deprivation and piealectomy. *Neuroendocrinology.* **42,**15-20

Blask, D.E. and Orstead, K.M. (1986) Dopamine inhibition of prolactin release but not synthesis in the male Syrian hamsters : *in vitro* studies. *Life Sciences.* **38,**1915-1921.

Blazej, A., Galatik, A., Galatik. J., Crul, Z. and Mladek, M. (1989) Atlas of microscopic structures of fur skins. pp135. Pub Elsevier

Burns, M. (1954) Observations on the development of the fleece and follicle population in Suffolk sheep. *J. Agric. Sci.* **44,**86-99

Burns, R.H., VonBergen, W. and Young, S.S. Cashmere and the undercoat of domestic and wild animals. *J. Text. Inst.* **53**(2),45-68

Buttle, H.L. (1974) Seasonal variation of prolactin in plasma of male goats. *J. Reprod. Fert.* **37**,95-99

Carter, H.B. (1943) Studies in the biology of the skin and fleece of sheep 1. The development and general histology of the follicle group in the skin of the

merino. *Bull. Coun. Scient. Ind. Res.,* Melb., No 164

Chase, H.B., Rauch, H. and Smith, V.W. (1951) Critical stages of hair development and pigmentation in the mouse. *Physiol. Zool.* **24**,1-8

Chase, H.B. (1954) Growth of the hair. *Physiol. Rev.* **34**,113-126

Chase, H.B. (1958) Physical factors which influence the growth of hair. In. *The Biology of Hair Growth* Eds W. Montagna and R.A. Ellis Chapter 17. Academic Press, N.Y. and London.

Chase, H.B. and Eaton, G.J. (1959) The growth of hair follicles in waves. *Ann. N.Y. Acad. Sci.* **83**,365-368

Chase, H.B. (1965) Cycles and waves of hair growth In. *Biology of the Skin and Hair Growth* Eds. A.G. Lyne and B.F. Short. pp461-466 Pub. Angus and Robertson Ltd. Sydney. Proc. Symposium Canberra Australia 1964.

Concannon, P.W., Weinstein, P., Whaleys. S. and Frank, D. (1987) Suppression of luteal function in dogs by luteinising hormone antiserum and by bromocriptine. *J. Reprod. Fert.* **81**,175-180

Curlewis, J.D., Loudon, A.S.I., Milne, J.A. and McNeilly, A.S. (1988) Effects of chronic long-acting bromocriptine treatment on liveweight, voluntary food intake, coat growth and breeding season in non-pregnant red deer hinds. *J. Endocrinology* **119**,413-420

Curlewis, J.D., Sibbald, A.M., Milne, J.A. and McNeilly, A.S. (1991) Chronic treatment with long-acting bromocriptine does not affect duration of the breeding season, voluntary food intake, body weight, or wool growth in the Scottish Blackface ewe. *Reprod. Fertil. Dev.* **3**,25-33

Dannies, P.S. (1982) Prolactin multiple intercellular processing routes plus several potential mechanisms for regulation. *Biochem. Pharmacol.* **31**(18), 2845-2849.

Diefenbach, P.W., Carmel, P.W., Frantz, A.G. and Ferin, M. (1976) Suppresion of prolactin secretion by L. dopa in the stalk sectional rhesus monkey. *J. Clin. Endocr. Metab.* **43**,638-642.

Doney, J.M. and Smith, W.F. (1969) Casting of the fleece in sheep. Estimation of experimentally induced fibre shedding rate. *J. Agric. Sci. Camb.* **73**,231-237.

Dry, F.W. (1926) The coat of the mouse (*Mus Musculus*). *J. Genet.* **16**,287-340.

Duncan, M.J. and Goldman, B.D. (1984) Hormonal regulation of the annual pelage colar cycle in the Djungarian Hamster, (*Phodopus Sungorus*). II. Role of prolactin. *J. Expt Zool.* **230**,97-103.

Ebling, F.J. and Hale, P.A. (1970) The control of the mammalian moult. *Mem. Soc. Endocrinol.* **18**,215-237.

Ebling F.J. and Johnson, E. (1961) Systemic influence on activity of hair follicles in skin homografts. *J. Embryol. exp. Morph.* **9**,285-293.

Eisemann, J.H., Bauman, D.E., Hogue, D.E. and Travis, H.F. (1984). Evaluation of a role for prolactin in growth and the photoperiod - induced growth response in sheep. *J. Anim. Sci.* **59**(1),86-94.

Ellis, R.A and Moretti, G. (1959). Vascular patterns associated with catagen hair follicles in the human scalp. *Ann. N.Y. Acad. Sci.* **83**,448-457.

Epstein, H. (1969) Domestic animals of china. Technical communication No. 18 of the Commonwealth Bureau of Animal Breeding and Genetics.

Everett, J.W. (1984). Luteotrophic function of autografts of the rat hypophysis. *Endocrinology.* **54**,685-690.

Ferguson, K.A. (1958) Proceedings of the New Zealand Society of Animal Production. **18**,128.

Ferguson, K.A., Wallace, A.L.C., and Lindner, H.R. (1965) Hormonal regulation of wool growth. In. *Biology of the skin and Hair Growth*. Ed. A.G. Lyne and B.F. Short. pp655-678. Publ. Angus and Robertson Ltd. Sydney. Proc. Symposium Canberra Australia 1964.

Fisher, D.A., Chapra, J.S., and Dussault, J.H. (1972). Extrathyroidal conversion of thyroxine to triiodothyronine in sheep. *Endocrinolgy*, **91**,1141-1144

Flux, J.E. (1970) Colour change of Mountain hares (*Lepus timidus scotious*) in North-East Scotland. *J. Zool. Lond.* **162**,345-358.

Forbes, J.M. (1982) Effects of lighting pattern on growth, lactation and food intake of sheep, cattle and deer. *Livestock Production Science.* **9**,361-374. Elsevier Scientific Publishing Co. Amsterdam.

Forbes, J.M. (1986) Environmental factors affecting intake. In. *The Voluntary Food Intake of Farm Animals.* Ed. J.M. Forbes Chapter 7, pp. 114-128. Pub. Butterworths and Co. Ltd.

Forsyth, I.A. (1983) The endocrinology of lactation. *Biochemistry of Lactation* Ed. T.B. Mepham. Chapter 10. pp.309-349. Elsevier Science Publishers B.V .

Forsyth, I.A., Byatt, J.C. and Iley, J. (1985) Hormone concentrations, mammary development and milk yield in goats given long term bromocriptine treatment in pregnancy. *J. Endocrinology* **104**,77-85.

Fraser. A.J. (1954) Development of the skin follicle population in merino sheep. *Aus. J. Agric. Res.* **5**,937-944.

Girard, J. (1969) Restauration par la thyroxine de la croissance de la laine supprime lors de l'hypophysectomie chez des brebis. Ile-de-France. *Annales de Biologie Animale Biochimie et Biophysique* **9**,497-512.

Hardy, M.H. (1951) The development of pelage hairs and vibrissae from skin in

tissue culture. *Annl. N.Y. Acad. Sci.* **53**,546.

Hardy, M.H. and Lyne, A.G (1956) The prenatal development of wool follicles in merino sheep. *Aus. J. Biol. Sci.* **9**,423.

Harris, D.R. (1962) The distribution and ancestry of the domestic goat. *Proc. of the Linnean Soc. of London.* **173**,79-91.

Hewson, R. (1958) Moults and winter whitening in the mountain hare (*Lepus timidus scatious*). *Proc. Zool. Soc. Lond.* **131**,99-108.

Holst, P.J., Clarke, W.H., and Maddocks, I.G. (1982) Skin and fleece characteristics of two groups of feral goats. *Aust. J. Exp. Agric. Anim. Husb.* **22**,173-176.

Hutchinson, J.C.D. (1965) Photoperiodic control of the annual rhythm of wool growth. In. *Biology of the Skin and Hair Growth.* Eds A.G. Lyne and B.F. Short, Chapter 34. pp 565-573. Pub. Angus and Robertson Ltd, Sydney. Proc. Symposium Canberra Australia, 1964.

Iason, G.R. and Mantecon, A.R. (1991) Seasonal variation in voluntary food intake and post-weaning growth in lambs: a comparison of genotypes. *Anim Prod.* **52**,279-286

Johnson, E. (1958) Quantitative studies of hair growth in the albina rat. I. Normal males and females. *J. Endocrinology.* **16**,337-350.

Johnson, E. (1977a) Seasonal changes in the skin of mammals. In. *Comp. Biol. of the Skin.* Ed. Spearman. Symp. Zool. Soc. Lond. No.39,379-404. Academic Press.

Johnson, E. (1977b) Cycles and patterns of hair growth. In. *The Physiology and Pathophysiology of the Skin. Vol IV. The Hair Follicle.* Ed. A. Jarrett. Chapter 1. Academic Press, London, N.Y., San Francisco.

Johnson, E. (1977c) The control of hair growth. In. *The Physiology and Pathophysiology of the Skin. Vol IV. The Hair Follicle.* Ed. A. Jarrett, Chapter 3. Academic Press, London, N.Y., San Francisco

Johnson, E. and Ebling, F.J. (1964) The effect of plucking hair during different phases of the follicular cycle. *J. Embryol. exp. Morph.* **12,**465-474.

Kanematsu, S. and Sawyer, C.H. (1973) Elevation of plasma prolactin after hypophysial stalk section in the rat. *Endocrinology.* **93,**238-241.

Kay, R.N.B. (1979) Seasonal changes of appeitite in deer and sheep. ARC. *Research Reviews.* **5,**13-15.

Kay, R.N.B. and Ryder, L.M. (1978) Coat growth in red deer (*Cervus elaphus*) exposed to a daylength cycle of six months duration. *J. Zool.* **185,**505-510.

Kay, R.N.B. (1983) Seasonal variation of appetite in ruminants. In. *Recent Advances in Animal Nutrition.* Ed. W Haresign. pp 113-129. Butterworths, London.

Kennaway, D.J., Gilmore, T.A, and Seamark, R.F. (1982) Effects of melatonin implants on the circadian rhythm of plasma melatonin and prolactin in sheep. *Endocrinology.* **110,**2186-2188.

Kennaway, D.J., Gilmore, T.A. and Seamark, R.F. (1982b) Effect of melatonin feeding on serum prolactin and gonadotrophin levels on the onset of seasonal estrous cyclicity in sheep. *Endocrinology.* **110,**1766-1777.

Kiyathkin, P.F. (1968) *Ways and methods of formation of a new breed of fibre-producing goats.* Tashkient, USSR, Uzbekistan, 264a.

Klein, D.C., Namboodiri, M.A. and Auerbach, D.A. (1981) The melatonin rhythm generating system; developmental aspects. *Life Sci.* **4,**20(10).

Klein, D.C., Namboodiri, M.A. and Sugden, D. (1983) 5-Hydroxytryptophan

elevates serum melatonin. *Science,* 12 August. 659-660.

Kligman. A.M. (1959) The human hair cycle. *J. Invest. Dermat.* **33**,307-316.

Labban, F.M. (1957) The effects of L-thyroxine on sheep and wool production. *J. Agric. Sci.* **49**,26-50

Lincoln, G.A., McNeilly, A.S. and Cameron, C.L. (1978) The effect of a sudden decrease or increase in daylength on prolactin secretion in the ram. *J. Reprod. Fert.* **52**,305-311.

Lincoln, G.A. (1984) The Pineal Gland. In. *Hormonal Control of Reproduction. Reproduction in Mammals Vol 3.* Eds. Austin & Short. pp. 52-75. 2nd Edition. Cambridge Univ. Press. London.

Lincoln, G.A. and Ebling, F.J.P. (1985) Effect of constant release implants of melatonin on seasonal cycles in reproduction, prolactin secretion and moulting in rams. *J. Reprod. Fert.* **73**,241-253.

Lincoln, G.A. (1990) Correlation with changes in horns and pelage, but not reproduction, of seasonal cycles in the secretion of prolactin in rams of wild, feral and domesticated breeds of sheep. *J. Reprod. Fert.* **90**,285-296.

Ling, J.K. (1970) Pelage and moulting in wild animals with special reference to aquatic forms. *Quart. Rev. Biol.* **45**,16-54.

Loudon, A.S.I., Curlewis, J.D., Milne, J.A. and McNeilly, A.S. (1989) A comparison of the seasonal changes and patterns of growth, voluntary food intake and reproduction in juvenile and adult red deer (*Cervus elaphus*) and Pere Davids deer (*Elephus Davidiarus*) hinds. *J. Endocrinology* **122**,733-745.

Lyne, A.G. (1966) The develpment of the hair follicle. Aust. J. Sci. **28**(10),370-377.

Lyne, A.G. and Heideman, M.J. (1989) The prenatal development of skin and

hair in cattle (*bos Taurus L.*). *Aus. J. Biol. Sci.* **12**,72.

Lyne, A.G. and Short, B.F. (1965) *Biology of the Skin and Hair Growth.* Pub. Angus and Robertson Ltd. Sydney. Proc. Symposium Canberra Australia 1964.

Maeda, K.I., Mori, Y. and Kano, Y. (1986) Superior cervical ganglionectomy prevevents gonadal regression and increases plasma prolactin concentrations induced by long days in goats. *J. Endocrinology* **110**,137-144.

Mantecon,A.R., Iason, G.R. and Hunter, E.A. (1989) Seasonal variation in voluntary feed intake in growing lambs: A comparison of genetypes. Asian J. Agric. Sci. **2**,284-286

Margolena, L.A. (1959) Skin and hair follicle development in dairy goats. The *Virginia Journal of Science. **10**,33-47*

Martinet, L., Meunier, M. and Allain, D. (1981) Control of delayed implantation and onset of spring moult in the mink (*mustela vison*) by daylight ratio, prolactin and melatonin. In. *Photoperiodism and Reproduction.* Nouzilly (France). Ed. INRA. Publ., (Les Colloques de l'INRA, 6).

Martinet, L., Allain, D. and Weiner, C. (1984) Role of prolactin in photoperiodic control of moulting in the mink (*mustela vison*). *J. Endocrinology.* **103**(1),9-15.

Martinet, L. and Allain, D. (1985) Role of the pineal gland in the photoperiodic control of reproductive and non-reproductive functions in mink (*mustela vison*) In. *Photoperiodism, Melatonin and the Pineal.* pp170-187. Pitman, London. Ciba foundation symposium 117.

Martinet, L., Allain, D. and Chabi, Y. (1985) Pineal denervation by cervical sympathetic ganglionectomy suppresses the role of photoperiod on pregnancy or pseudo pregnancy, body weight and moulting periods in the mink (*mustela vison*). *J. Endocrinology.* **107**,31-39.

Mercer, E.H. (1961) *Keratin and Keratinization: An Essay in molecular biology*. Pergamon Press, Oxford.

Millar, P. (1986) The performance of Cashmere Goats. *Animal breeding Abstracts*. **54.** 1986. No.3.

Milne, J.A., Loudon, A.S.I., Sibbald, A.M., Curlewis, J.D. and McNeilly, A.S. (1990) Effects of melatonin and a dopamine agonist and antagonist on seasonal changes in voluntary intake, reproductive activity and plasma concentrations of prolactin and tri-iodothyronine in red deer hinds. *J. Endocrinology.* **125,**241-249.

Montagna, W. (1956) The structure and function of skin. *Acad. Press. Inc.* N.Y.

Moore, R.Y. (1978) The innervation of the mammalian pineal gland. *Prog. Reprod. Biol.* **4,**1-29.

Mori, Y., Maeda, K.I., Sawasaki, T. and Kano, Y. (1985) Photoperiodic control of prolactin secretion in the goat. *Jpn. J. Anim. Reprod.* **31,**9-15

Muduuli, D.S., Sanford, L.M., Palmer, W.M. and Hewland, B.E. (1979) Secretory patterns and circadian and seasonal changes in luteinizing hormone, follicle stimulating hormone, prolactin and testosterone in the male pygmy goat. *J.Anim. Sci.* **49**(2),543-553.

Muir, L.A., Hibbs, J.W., Conrad, H.R. and Smith, K.L. (1972) Effect of estrogen and progesterone on feed intake and hydroxyproline excretion following induced hypocalcaemia in cows. *J. Dairy Science.* **55,**1613-1620.

Munro, D.D. (1956) Hair growth measurement using 35s cystine. *Archs. Derm.* **93,**119.

McDonald, B.J. (1985) The cashmere growth cycle. *Proc. of 1st International Cashmere Seminar.* pp 125-133. March 1985.

McDonald, B.J., Hoey, W.A. and Hopkins, P.S. (1987) Cyclical fleece growth in cashmere goats. *Aust. J. Agric. Res.* **38**,597-609.

McLeod, R.M. and Robyn, C. (1977) On the mechanism of increased prolactin secretion by sulpiride. *Endocrinology* **72**,273-277.

McNeilly, A.S. and Andrews, P. (1974) Purification and characterization of caprine prolactin. *J. Endocrinology* **115**,273-282.

Niklowitz, P., and Hoffmann,K. (1986) Involvement of the pituitary in photoperiod induce changes of coat colour and body weight in the Djungarian hamster. *Biol. Reprod.* **34**(Suppl 1.):67.

Niklowitz, P. and Hoffmann, K. (1988) Pineal and pituitary involvement in the photoperiodical regulation of body weight coat colour, and testicular size of the Djungarian hamster, *Phodopus sungorus. Biology of Reproduction.* **39**,489-498.

Nixon, A.J., Gumsey, M.P., Betteridge, K., Mitchell, R.J. and Welch, R.A.J. (1991) Seasonal hair follicle activity and fibre growth in some New Zealand cashmere-bearing goats. *J. Zool.(London).* In press.

Oliver, R.F. (1969) The vibrissa dermal papilla and its influence of epidermal tissues. *Br. J. Derm.* **81**,55.

Oliver, R.F. and Jahoda, C.A.B. (1989) The dermal papilla and maintenance of hair growth. In: *The Biology of Wool and Hair.* Ed. Rogers, G.F., Reis, P.J., Ward, K.A. and Marshall, R.C. London. N.Y. (Chapman and Hall).

Orstead, K.M. and Blask, D.E. (1987) Neuroendocrine effects of light deprivation and pinealectomy *in vivo* on the time course of changes in prolactin cell activity *in vitro. Neuroendocrinology.* **45**(3),182-190.

Panaretto. B.A. (1979) Effect of light on cyclic activity of wool follicles and possible relationship to changes in the pelage of other mammals. In.

Physiological and Environmental Limitations to Wool Growth. Ed. J.L. Black and P.J. Reis. Univ. of N. England Publishing Unit.

Pelletier, J. (1973) Evidence for photoperiodic control of prolactin release in rams. *J. Reprod. Fert.* **35**,143-147.

Pinkus, H. (1958) Embryology of hair. In. *The Biology of Hair Growth*. Chapter 1. Eds. Montagna, W. and Ellis, R.A. Academic Press, N.Y. and London.

Plotka, E.D., Seal, U.S. and Verme, L.J. (1982) Morphological and metabolic consequences of pinealectomy in deer. In. *The Pineal Gland, Vol III, Extra-reproductive effects*. Ed. Reiter, R.J. CRC Press, Boca Raton, Florida,

Quay, W.B. (1963) Circadian rhythm in rat pineal serotonin and its modifications by estrous cycle and photoperiod. *Gen. Comp. Endoc.* **3**,473-479.

Quay, W.B. (1964) Circadian and estrous rhythms in pineal melatonin and 5-Hydroxy Indole-3-Acetic Acid. *Proc. Soc. Exp. Biol. Med.* **115**,710-713

Ravault, J.P. (1976) Prolactin in the ram: seasonal variations in the concentrations of blood plasma from birth until three years old. *Acta Endocrinol.* **83**,720-725.

Ravault, J.P, and Ortavant, R. (1977) Light control of prolactin secretion in sheep. Evidence for a photo-inducible phase during a diurnal rhythm. *Annales de Biologie Animale. Biochimie et Biophysique.* **17**,459-473.

Reklewska, B. Total serum thyroxine concentration during ontogenic development of sheep. *Acta. Physiologica Polonica.* **26**,462.

Roberts, T.J. (1969) A note on *capra falconeri* -(Wagner 1839). *Zeitschrift fur Saugetierkunde.* **34**, 228-249.

Robyn, C. *Interrelations between prolactin and the hypothalamo-hypophysio-thyroid axis: a review*. Ist. Int. Symp. on basic Applications and Clinical Use of Hypothalamic Hormones. Eds. A.L. Charro-Salgado, R. Fernandez-Durango, and G.Lopez del Campo. pp319-342. Excerpta Medica (I.C.S. No.374) Amsterdam.

Rodgers, L. (1990) Management of cashmere goats. In. *Scottish Cashmere - the Viable Alternative.* Ed. Russel, A.J.F. Scottish Cashmere Producers Assoc.

Rollag, M.P., O'Callaghan, P.L. and Niswender, G.D. (1978) Serum melatonin during different stages of the annual reproductive cycle in ewes. *Biol. Reprod.* **18**, 279-285.

Rolland, R., Nijdan, W., Weyer, A. and Lancranjan, I. (1986) Prevention of puerperal lactation with Parlodel Long-Acting. (Parlodel.L.A.) Eur. J. Obstet. Gynecol. *Reprod. Biol.* **22**,337-343.

Rose, J., Stormshak, F., Oldfield,J. and Adair, J. (1985) The effects of photoperiod and melatonin on serum prolactin levels of mink during the autumn moult. *J. Pineal Res*. **2**,13-19.

Rougeot, J. (1961) Comparative effects of annual and semi-annual periodic variations of day length on the follicle cycles of the short kemp fibres of the fleece of Limousine ewes. Relationship with reproductive cycles. *Annales de Biologie Animale Biochimie et Biophysique* **1**,385-402.

Rougeot, J. Thebault, R.G. and Allain, D. (1984) Role of the compound hair follicle in adaptive pelage changes. *Acta. Zool. Fennica.* **171**,19-21.

Rusak, B., and Zucker, I. (1979) Neural regulation of circadian rhythms. Physiol. Rev. **59**(3),449-526.

Rust, C.C. (1962) Temperature as a modifying factor in the spring pelage change of short-tailed weasels. *J. Mammol.* **43**,323-328.

Rust, C.C. (1965) Hormonal control of pelage cycles in the short-tailed weasel (*Mustela erminea bangsi*). *Gen and Comp. Endocr.* **5**,221-231.

Rust, C.C. and Meyer, R.K. (1968). Effects of pituitary autografts on hair colour in the short-tailed weasel. *Gen. and Comp. Endocr.* **II**,548-551.

Ryder, M.L. (1960) A study of the coat of the mouflon (*Ovis Musiman*) with special reference to seasonal change. *Proc. Zool. Soc. Lond.* **135**,387-404.

Ryder, M.L. (1966) Coat structure and seasonal shedding in goats. *Anim. Prod.* **8**,289-302.

Ryder, M.L., and Stephenson, S.K. (1968) *Wool Growth.* Academic Press, London. N.Y.

Ryder, M.L. (1969) The development and structure of, and seasonal change in, the coat of some Wiltshire sheep. *Anim. Prod.* **II**,467.

Ryder, M.L. (1971) Wool growth cycles in soay sheep. *J. Agric. Sci. Camb.* **76**,183-197.

Ryder, M.L. (1973) The structure of, and growth cycles in, the coat of wild mouflon sheep (*Ovis musiman*) and their crosses. *Res. Vet. Sci.* **15**,186-196.

Ryder, M.L. (1984) Prospects for cashmere production in Scotland. *Wool Record. (November)* **37**,43.

Sar, M., Sar, A.H., Sar, M.S. and Calhoun, M.L. (1966) Microscopic anatomy of the integument of the common American goat. *Am. J. Vet.* Res. **27**(117),444-456.

Serron-Ferre, M., Vergara, M., Parraguez, V.H., Riguelme, R. and Llanas, A.J. (1989) Fetal prolactin levels respond to a maternal melatonin implant. *Endocrinology* **125**,400-403.

Schanbacher, B.D. and Crouse, J.D. (1981) Photoperiodic regulation of growth - a photosensitive phase during the light-dark cycle. *Am. J. Physiol.* **241**,E1-E5.

Schinckel, P.G. (1953) Follicle development in the Australian merino. *Nature, Lond.* **171**, 310-311.

Short, B.F. (1955) Development of the secondary follicle population in sheep. *Aus. J. Agric. Res.* **6**,62-67.

Slee, J. (1959) Fleece shedding, staple-length and fleece weight in experimental Wiltshire Horn-Scottish Blackface crosses. *J. Agric. Sci. Camb.* **3**,209-233.

Slee, J. (1965) Seasonal patterns of moulting in Wiltshire horn sheep. In. *Biology of the Skin and Hair.* Eds. Lyne, A.G. and Short, B.F. pp 543-563. Angus and Robertson, Sydney.

Smith, A.J., Mondain-Monval, M., Andersen-Berg, K, Simon, P., Forsberg, M., Clausen, O.P.F., Hansen, T., Moller, O.M. and Scholler, R. Effects of melatonin implantation on spermatogenesis. The moulting cycle and plasma concentrations of the male blue fox (*Alopex Lagopus*). *J. Reprod. Fert.* **79**,379-370.

Smith, A.J., Mondain-Monval, M., Simon, P., Andersen-Berg, K., Clausen, O.P.F., Hofmo, P.O. and Scholler, R. (1987) Preliminary studies of the effects of bromocriptine on testicular regression and the spring moult in a seasonal breeder, the male blue fox (*Alopex lagopus*). *J. Reprod. Fert.* **81**,517-524.

Sokal, R.R., and Rohlf, F.J. *Biometry. The Principles and Practice of Statistics in Biological Research. 2nd. Edn.* W.H. Freeman and Co. San Francisco.

Spearman, R.I.C. (1964) The evolution of mammalian keratinized structure. In The Mammalian Epidermis and its Derivatives. *Symp. of the Zoological Soc. of London,* **12**,67 Ed. Ebling, F.J.G. Academic Press. London and N.Y.

Spearman, R.I.C. (1977a) Hair follicle development, cyclical changes and formation. In. *The Physiology and Pathophysiology of the Skin, Vol IV. The Hair Follicle.* Ed. Jarrett, A. Chapt 2. Academic Press. London, N.Y., San Francisco.

Spearman, R.I.C. (1977b) The structure and function of the fully developed follicle. In. *The Physiology and Pathophysiology of the Skin, Vol IV. The Hair Follicle.* Ed. Jarrett, A. Chapter 4. Academic Press, London, N.Y., San Francisco.

Stetson, M.H., Elliot, J.A. and Goldman, B.D. (1986) Maternal transfer of photoperiodic information influences the photoperiodic response of prepubertal Djungarian hamsters. *Biology of Reproduction.* **34**,664-669.

Straile, W.E., Chase, H.B. and Arsenault, C. (1961) Growth and differentiation of hair follicles between periods of activity and quiescence. *J. Exp. Zool.* **148**,205-221.

Straile, W.E. (1965) Root sheath-dermal papilla relationships and the control of hair growth. *Biology of the Skin and Hair Growth.* Eds. Lyne A.G. and Short, B.F. Pub. Angus and Robertson Ltd. Sydney. Proc. Symposium Canberra, Australia.

Talwalker, P.K., Ratner, A. and Meites, J. (1963) *In vitro* inhibition of pituitary prolactin synthesis and release by hypothalamic extracts. *Am. J. Physiol.* **205**,213-218

Tamarkin, L., Baird, C.J. and Almeida, O.F.X. (1985) Melatonin: A coordinating signal for mammalian reproduction? *Science* 15 Feb.

Theriez, C. and Rougeot, J. (1962) Action des hormones thyroidennes sur la croissance on longueuer du brin de laine. *Annales de Biologie Animale Biochimie Biophysique* **2**,5-11

Van Scott, F.J., Ekel, T.M., and Auerbach, R. (1963) Determinants of rate and kinetics of cell division in scalp hair. *J. Invest. Derm.* **31**,281-287.

Vriend, J. (1983) Pineal-thyroid interactions. *Pineal Research Reviews.* **1**,183-206.

Wallace, A.L.C. (1979a) The effect of hormones on wool growth. In. *Physiological and Environmental Limitations to Wool Growth.* Eds. J.L. Black and P.J. Reis. Univ. of New England. NSW Australia.

Wallace, A.L.C. (1979b) Variations in plasma thyroxine concentrations throughout one year in penned sheep on a uniform feed intake. *Aust. J. Biol. Sci.* **32**,371-374.

Watson, A. (1965) The effect of climate on the colour change on mountain hares in Scotland. *Proc. Zool. Soc. Lond.* **141**,823-835.

PREPARATION OF SKIN BIOPSIES FOR HISTOLOGICAL EXAMINATION

Removal and fixing of biopsy

An area of skin is clipped using surgical clippers, and cleaned with surgical disinfectant. 1-2ml of local anaesthetic (Lignocaine or Lignovet, Dunlop Veterinary Suppliers) is injected under the dermis until a bubble is formed. A piece of skin (about 1 cm diameter) is removed with sharp curved scissors. The wound is sprayed with oxytetrin (Dunlop Veterinary Suppliers). The biopsy is pressed flat on to card and placed into 10% buffered formal saline.

The samples are fixed in formal saline for a minimum of 48 hours. They are then trimmed and the excess hair removed prior to processing.

Tissue Processing

The samples are placed into a histokinet and immersed in a series of solutions which dehydrate the tissue and then cleared with a solution that is miscible with paraffin wax, eg 'Histoclear' (a xylene substitute).

The standard procedure is outline below:

Bath	**Solution**	**Time** (hours)
1	10% buffered formal saline	1.0
2	50% Alcohol	3.0
3	70% "	3.0
4	90% "	1.5
5	90% "	1.5
6	Absolute Alcohol	0.5
7	Absolute Alcohol	0.5
8	Carbolic histoclear	3.0
9	Histoclear	1.5
10	Histoclear	0.5
11	Paraffin wax (60°C)	1.5
12	Paraffin wax (60°C)	1.0

The sample is embedded in wax under reduced pressure and then placed into a mould, hair side down and pressed flat on to a marble slab.

Sectioning

Excess wax is trimmed from the sample which is then mounted on a wooden chuck with molten wax. The sample is placed exactly vertical to the microtome blade and sections of 8 microns are cut. The sections are mounted on to glycerin albumen coated slides and dried at 28°C overnight.

Staining

The dehydrated mounted sections are placed through a series of alcohols and stains to selectively and differentially stain areas of the hair follicles, accessory structures and connective tissue. The process is outlined below.

Step	Solution	Time (min)	Comments
1 & 2	Histoclear	2 x 5	removes wax
3	Absolute alcohol	2	removes histoclear
4	94% Alcohol	3	gently rehydrates to level of stain 5
5	Weigharts haematoxylin	11	nuclear stain
6 & 7	Wash	2 x 1	differential staining to level required
8	Acid alcohol	1	
9	Wash	1	
10	50% Alcohol	2	Rehydration
11	Basic Fuchsin	4	stains ONA
12	Wash	3	
13, 14&15	50% Alcohol, 70 % Alcohol, 90 % Alcohol	3 x 2	Dehydration
16	Picric acid	25	lightens nuclei
17	70% Alcohol	2	Rehydration
18	Distilled water	2	
19	Picro-indigo-carmine	4	Stain collagen and cytoplasm
20	50% Alcohol	2	Dehydration

21	70% Alcohol	2	
22	Absolute Alcohol	1	
23	Carbolic histoclear		
24	Histoclear	4	removes alcohol

This results in yellow cytoplasm and blue and blue-green collagen. When PIC is used after basic fuchsin this is known as Cajals trichome.

Mounting

The slides are dipped into histoclear/CNP30 and coverslips mounted with DePe X (BDH).

The slides are examined by light microscopy in normal field and follicles identified at x 40 magnification.

QUALITY CONTROL MEASURES USED IN RADIOIMMUNOASSAY OF PLASMA PROLACTIN CONCENTRATIONS

Quality controls are employed to monitor the precision of an assay system and to ensure reproductability both within an assay and between assays.

Preparation of quality controls

Animals known to be high or low in plasma prolactin concentration (ie long or short photoperiod) were bled, and the plasma pooled and aliquotted into high and low quality controls. The medium controls were obtained from mixed high and low plasma to obtain a mid-range concentration.

The quality control standards were placed in triplicate every 200 samples immediately subsequent to the standard curve.

Intra-assay variation

If the reproducability of each individual assay (coefficient of variation of the results of the quality control samples) is not less than 12.0 % then the assay is rejected and repeated.

Inter-assay variation

If any of the controls, when combined with the controls of other assays, increases the coefficient of variation to more than 14.0%, then the assay is rejected and repeated.

Assay drift

Standards in triplicate were placed every 200 samples to compensate for assay drift.

Range of concentrations

The range of standards was from 5 ng/ml to 250 ng/ml. If any sample was equal or greater than 250 ng/ml then it was diluted 1:1 with buffer and repeated.

The lower limit of detection of the assay was 0.4 ng/ml.